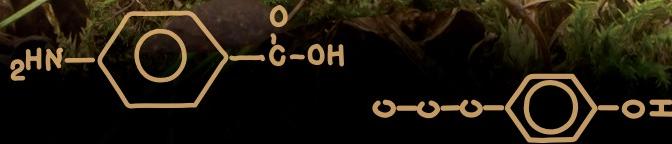


Second Edition

HUMIC MATTER IN SOIL AND THE ENVIRONMENT

Principles and
Controversies

Kim H. Tan



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Controversies

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Crops and Soil Science, University of
Georgia, Athens, Georgia, USA



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Preface

Since the first edition was printed in 2003 by Marcel Dekker Inc., *Humic Matter in Soil and the Environment* is the only book of its kind addressing specifically humic acid, its principles, and its controversies. As is often underscored, other books, although of utmost importance for the scientific dissemination of knowledge in humic acid chemistry, are mostly conference proceedings examining specialty topics only. Hence, the first edition seems to have been received well by professionals, scholars, and other readers alike who needed a coherent assessment of the principles and basic understanding of humic acid and its chemistry, as evidenced by the five-star ratings of the book by both Amazon and Google.

At the time the first edition went into circulation, the world of humic matter was experiencing drastic changes in concepts and principles. The biopolymer theory, which for almost two centuries had been the pillar of humic acid science, was losing ground because of its alleged failure to provide consensus on a commonly accepted molecular weight and the like. The operational concept, introduced by some of the founding members of the International Humic Substances Society (IHSS) to replace the biopolymer theory, failed to materialize as a major concept. However, the emergence of the supramolecular chemistry concept near the start of the new millennium has brought new ideas of small organic molecules self-assembling into humic substances. Although it escaped attention at the start, a couple of years later, owing to multiple publications, the supramolecular chemistry concept was considered as a significant change in the concept of humic acids. However, the basis of this new theory was, in fact, not new but was preceded by an earlier excellent concept based on similar principles called the micelle or micellar theory, which had been buried in the maze of literature references and brought to light from apparent obscurity because of the sudden interest in the humic self-assemblage theories. Very recently, in 2012, the present author added new changes by introducing his nanotube concept,

followed in 2013 by his humo–nanotube membrane concept, bringing the theories of small molecular humic assemblages to nanolevels. In contrast to the supramolecular idea allegedly derived from a review of the literature and from conjecture, the nanotube and nanotube membrane hypotheses were supported by scanning electron microscopy research showing concrete evidence of the presence of nanotube and nanotube membrane structures in humic molecules extracted from a variety of soils.

Other notable changes occurring at that time were related to the rapid development of the science of aquatic organic matter. Spearheaded by geologists, geochemists, hydrologists, and some limnologists—who temporarily took over the initiative of humic acid research from soil scientists—the science of aquatic organic matter has reached the current eminent scientific status within only a couple of years. Exemplified by algae, plankton, and kelp from both freshwater and marine ecosystems, this group of aquatic organic matter is distinctly different by being more aliphatic and carbohydrate in nature than its terrestrial counterpart, which is predominantly composed of lignin and aromatics. These differences will have a significant effect on the formation of aquatic humic matter, which the author proves in this book to be different in composition and structure from terrestrial humic substances.

All the new ideas mentioned above, as well as other developments, necessitate issuing a second edition to cope with changes and in order to move forward with the advances in modern humic acid chemistry of the new millennium. The concept of organic matter has to be revised and adjusted to new ideas, which warrant a distinction into three separate categories. The general term “organic matter” should be perceived as the highest category, encompassing all organic matter in the Earth’s ecosystem, followed by the next lower category called “humus,” the product of decomposition. Humus is composed of a nonhumified and a humified fraction; the humified fraction, called “humic matter” by the author, constitutes then the third (lowest) category of organic matter. The above is then the start and part of the approach for a rationale in writing this second edition, with Chapter 1 examining organic matter as a whole; Chapter 2 studying the concept of humus, focusing on the nonhumified fraction, which is composed essentially of plant biopolymers in their original or slightly decomposed forms; and Chapter 3 specifically relating the concepts of humic matter—that is, what one is talking about when referring to humic acid. Even now, the term “humic acid” is still controversial, and it depends on the specific concept and/or person. The remaining chapters are then about the nature and distribution, genesis, extraction, chemical composition, characterization,

electrochemical properties and reactions, and agronomic and industrial applications, including medicinal and pharmaceutical applications, of humic substances.

Chapters

This second edition is essentially a whole new version and is composed of eleven new and rewritten chapters, as opposed to nine chapters in the first edition. These eleven chapters are written from new materials, using the old text as a basic framework only. Hence, the new eleven chapters cannot be equated with the nine older versions. A chapter-wise breakdown of new advances and of the changes to the earlier editions is provided in some detail.

Chapter 1, examining organic matter at the highest category, is essentially a completely new chapter. It is now composed of two expanded main sections: (1) terrestrial organic matter and (2) aquatic organic matter, in line with the new developments. A section on “extraterrestrial organic matter” is added for completeness. The issue of biomass is expanded and is now divided into (1) terrestrial, (2) aquatic, and (3) industrial biomass. Each of the above concepts is properly identified or delineated by prevailing definitions. A pedological concept of organic matter, called SOM (soil organic matter), and issues of DOM (dissolved organic matter) as operational compounds are also provided. The importance of these different categories of organic matter and their biomasses, their functions in trophism, and how these differences affect organic matter or organic carbon accumulation are examined. Due attention has also been directed to issues on differences of decomposition (aerobic versus anaerobic) and the effects on ecology. Changes in water chemistry, given here the name *hydrochemistry*, and *photochemical bleaching* are addressed, among others. A variety of acronyms used in the identification of humic substances, such as NOM and DOM, are discussed, and a table listing the most commonly used acronyms is provided for a handy reference.

Chapter 2 is about humus, with the mission to underscore the message that it is the decomposition product of organic matter composed of nonhumified and humified materials. The nonhumified components—making up the bulk of the text—are important for the formation of the humified fraction. They are essentially the biopolymers released, by decomposition, from the plant tissue in their original or in broken forms, such as carbohydrates, lignin, protein, and lipids. Aromatics formed by aromatization of sugars and phenols,

and quinones produced by alteration of lignin and other aromatics, such as pyrogallol, are included, and the formation processes are illustrated by drawings (figures) and explained in the text. Their functions and importance as precursors or raw materials for the formation of humic matter are presented in simple language. For a complete list of the plant polymers covered, including xenobiotics, see the Table of Contents. The humified fraction is covered as the alkali-soluble portion of humus at the end of this chapter, which serves mainly as an introduction to Chapter 3 because this is, in essence, humic matter.

Chapter 3 is a reconstruction of the concepts of humic matter, the third and lowest category of organic matter. It is, in essence, a brand new chapter covering all concepts from the biopolymer, the oldest and most trusted view of humic acid, to the latest ideas based on supramolecular chemistry. A hot topic—the *in vivo* humification hypothesis—is included, which suggests the controversial idea of humic substances being produced within the plant body. The issues and controversies of the understanding on humic matter according to the biopolymer, operational, micellar, supramolecular, nanotube, and nanotube membrane concepts are addressed in some detail. Scanning electron micrographs are presented to evidence the existence of carbon nanotube structures and of hexagonal structures in the form of honeycombs characteristic of the presence of nanotube membranes in humic molecules. The theory of dimensionless micelles is corrected by the author by imposing minimum sizes of 2–10 nm, the nanometer length of an amphiphile. The issues on molecular weights and HPSEC (high-pressure size exclusion chromatography), the prime basis for the introduction of the supramolecular concept, are examined, with possible revisions for improvements suggested.

Chapter 4 is about the nature and distribution of humic matter. It is revised and expanded to also cover the nature of humic acid as cell sap of ulm (elm) trees, hence the name ulmic acid in the old German literature. During the early periods, leaf mold and *torf*—old German for *peat*, but mistakenly translated as *turf* in many books—have also been used for humic acid extraction, and the extracted compounds identified by a variety of names, such as geine, geic acid, crenic and apocrenic acids, which are confusing for both the early researchers and modern scientists. The nature of humic matter in the early twentieth century and its new developments at the turn of this century are discussed in some detail. Owing to its increased importance in industry, humic matter in geologic deposits is expanded to include kerogen and shilajit. Kerogen is the raw material for asphalt, whereas shilajit (a Sanskrit name), distributed extensively in the Altai and

Tibet mountains of the Himalayas, has attracted considerable attention in the pharmaceutical industry for its use in alternative medicine as anti-inflammatory, anti-cancer, anti-stress, and anti-aging agents.

Chapter 5 discusses only the major pathways, and the humification theories are expanded by addition of several other authentic versions of the theories, making the chapter also brand new. The types and chemistry of plant biopolymers are more closely related to the topic of the nonhumified organic matter and are now discussed in Chapter 2. Kononova's phenol theory, the benzene carboxylic hypothesis of Schnitzer and Khan, and Stevenson's phenol dimer theory are added for comparison to the ligno-protein, phenol-protein, and sugar-amine condensation theories previously covered, presenting at the same time the issues and controversies accompanying the comparative evaluation examinations. The more modern micellar hypothesis of Wershaw, Piccolo's supramolecular concept, and the humo-nanotube membrane theory of the present author constitute the key topics of Chapter 5. The issue of humification versus diagenesis—a geological generic name for humification—is examined and clarified. The terms "diagenesis" and "geopolymers" are often used today by geologists and geochemists. They are considered "migrants," imported by force from geology into soil science. The issue of biopolymer degradation versus abiotic condensation pathways is addressed.

Chapter 6 examines the extraction, isolation, and fractionation of humic matter. Ignoring the extensive rewrite and several major additions, this is perhaps the only chapter carrying over most of the text of the first edition (formerly Chapter 3), because most of the procedures for extraction of terrestrial as well as aquatic humic matter are still up to date. The definitions of the isolated product as humic acids, fulvic acids, humin, hymatomelanic acid, and brown and gray humic acid fractions are still in use today. Explicit instructions provided on the standard NaOH extraction are virtually similar to the Soil Science Society of America and the IHSS procedures. The issue of using a nitrogen atmosphere during extraction is examined. Extraction of aquatic humic matter with the XAD resin method is provided separately in some detail. Fractionation into several types of humic compounds and fractioning into size fractions (molecular weights) are presented. Microfiltration and ultrafiltration techniques are assessed. Various reagents, e.g., Sephadex, other types of resins, and glass beads are examined in gel filtration, called today size exclusion chromatography or SEC. Differences in LPSEC (low performance) and HPSEC (high performance) and the new (2013) ultra-HPSEC methods are addressed. The issue of isolation of DOM and limits of the 0.45 µm filter requirement are discussed. The difference with DOC is

underscored, and a microanalytical method for measuring DOC directly in water by a carbon analyzer is provided as a new technique.

Chapter 7 on chemical composition and model structures of humic acids is completely revised, reorganized, and expanded to include the more recent (2012) hypothesis of internal oxidation (ω) by Dębska and coworkers. The significance of the effect of cultivation on the change in values of ω , as reported by Dębska in an earlier 2009 investigation, is addressed. The theory of minimum formula (molecular) weight is revised, adding fresh ideas to the chapter. Its calculations using several hypothetical humic acids containing 2–30 N are shown to produce at 30 N a minimum molecular weight of 10,690 corresponding to a formula of $C_{510}H_{510}O_{240}N_{30}$, and discussed to be a value closely similar to that of low molecular weight humic acids extracted from natural soils. The issue of aliphatic humic matter, currently receiving increased attention, as compared with aromatic humic substances is discussed and evaluated. Model structures based on the lignin monomer concept of Flaig, lignin tetramer concept of Steelink, phenol oligomer concept of Schulten, phenol polymer concept of Schnitzer and Orlov, and several others, are highlighted, and the accompanying issues are presented for readers to evaluate.

Chapter 8 is about the chemical and spectroscopic characterization of humic substances. As with other chapters, this is completely revised and expanded to include current advances and issues. The alleged absence of a common molecular weight of humic acid is reassessed by using new data from Song and coworkers published in 2010 and Perminova and coworkers in 2003, the latter perceived to be the leading authorities on the subject in Russia. The use of molecular weight for characterization of humic molecules is studied, and limitations of analyses and calculations, including the controversy on using daltons and dimensionless units, are presented. The use of LPSEC and HPSEC as the bases for creating the supramolecular self-assemblage concept is studied and found to be controversial because of the production of fake size fractions, called “operational” fractions, as stated earlier in Chapter 6. Humic fractions at any size limit produced by the analysis depending on the Sephadex used in the chromatographic column are discussed as major concerns. Reassessment of interpretation of scanning electron micrographs provides stunning revelations about the existence of nanotube membrane structures in humic molecules, supported by the added new (2013) SEM pictures. All these changes make this second-edition chapter outshine the first-edition version, meeting the challenges created by the advancing knowledge of the new millennium.

Chapter 9 addresses the electrochemical properties of humic matter. The changes, revisions, and additions to update the older version make this essentially a new chapter. The amphoteric, Brønsted–Lowry, and Lewis acid–base concepts are now applied to explain the origin and types of electro-negative charges of humic substances. The importance of functional groups as creators of the negative charges is discussed in some detail in relation to dissociation of protons as affected by pH. Selected data from an IHSS 2003 report are discussed, revealing the dominant effect of carboxyl (COOH) group contents. A discussion and examples of calculations are provided to predict the amount of negative charges created by the dissociation of COOH groups using the pK_a relationship and the Henderson–Hasselbalch equation. The double-layer concepts, adsorption, cation exchange, and chelation are revised, reorganized, or revamped to cover current advances. The bridging mechanism is now the focus and discussed as the key issue for the complex reaction capacity of humic acids. Water-bridging and cation-bridging reactions are discussed as the more common interaction reactions of humic substances.

Chapter 10 examines the agronomic importance of humic matter. Issues on the indirect and direct effects of humic matter on plant growth in general and crop production specifically are rewritten and expanded substantially. Issues on soil structure, soil texture, and bulk density as affected by humic matter are added. The significance of soil structure formation is linked to the more modern supramolecular chemistry concept. Self-assemblage of soil particles is assessed as a more significant process in soil structure formation than traditional aggregation aided by cementation effects of organic matter. Application of the humo–nanotube concept is discussed, allowing tailoring of soil research to structure formation at the nanoscale and microscale, where the principles of the phenomena are initiated. The author argues that it is nanoscience, as covered in Chapter 3, but applied here in soil structure formation at a very small scale. Water and metal bridging are discussed as the simplest reaction processes linking the soil particles together into thermodynamic stable associations. The other changes, e.g., on soil consistency by involving Atterberg's plastic and liquid limits, are focused on soil stability and erosion. The effect on soil chemical properties is addressed on charge properties produced by the functional groups of humic matter as related to pH– pK relations. Chelation by humic acids of xenobiotics and other soil contaminants is expressed as an issue of soil hygiene. Misinterpretations of the redox concept by some chemists or geochemists, providing improper education to readers, are corrected by the author, and the providing of improper

education to readers due to dissemination of misinformation is replaced by the proper reaction equations. The concept of redox reactions is addressed also as a photochemical reaction, and the concept of reduced and oxidized forms of humic acids addressed as controversial issues. All the above revisions, including those related to the biological properties of soil, improve the first-edition version in terms of both quantity and quality.

Chapter 11 is about the environmental and industrial importance, including pharmaceutical, of humic matter. This second-edition version is also revised, rewritten, and expanded considerably, necessitating the author to focus the readers' attention on only a few major revisions. The significance of humic acid in the accumulation and preservation of soil organic matter is discussed using two examples of the most fertile soils in the world, the mollisols with high contents in Ca humate and the andosols rich in Al humates. The controversy of blaming shifting cultivation for soil degradation due to rapid loss of SOM is discussed, and identifying shifting cultivation as synonymous to the slash-and-burn technique is addressed as incorrect. Biological detoxification is considered to be the use of humic acids as eco-friendly detoxifiers. The importance of humic substances in industry is addressed in relation to increased production of humates by dry processing of lignite. The issue of mixing humates with microorganisms and saponin is examined, and suggestions are presented. The production of bio-superphosphates and other agrochemicals for use as fertilizers, and the production of silicone from humic acids for use as lubricants and breast implants are discussed. The importance of humic substances in the pharmaceutical industry is also provided with the most recent information on advances in medical science. Their significance as pharmaceuticals starts with the balneotherapeutic use of peat, which is now traced to the active component of peat, humic acid. Scientific research at various hospitals and clinics in Germany is discussed to reveal the therapeutic features of humic acids as analgesic, antiviral, anti-inflammatory, and lipid-modulatory agents. Results of clinical studies by German physicians as recently as 2005 are discussed, suggesting humic acid reagents to be effective against the influenza A virus and the herpes simplex virus. The latter is addressed in the present book as the reason for the start of the commercial production of a lipstick containing humic acids to prevent herpes infection. The issues of mutagenesis and the blackfoot disease, implicating humic acids as a possible cause, are assessed.

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Inc., and an international panel member of the *Malaysian Journal of Soil Science*. His most popular book, *Principles of Soil Chemistry*, is now in the fourth edition, and his *Environmental Soil Science* book was recently published in 2009 in its third edition by Taylor & Francis/CRC Press. Another one of his important books is *Andosols*, a Hutchinson Ross benchmark book by Van Nostrand Reinhold Co., which was sold out as soon as it was published.

Dr. Tan earned an MS degree (1955) in agronomy from the University of Indonesia and graduated with a PhD (1958) in pedology under Dr. Ir. J. Van Schuylenborgh, past deacon of the subfaculty of the University of Amsterdam, Amsterdam, the Netherlands. After postdoctoral training at North Carolina State University, Raleigh, North Carolina, and Cornell University, Ithaca, New York, under the tutorship of Drs. Ralph McCracken, Adolf Mehlich, Marlin Cline, and Michael Peech, he returned to the University of Indonesia, where, as the head of the Department of Soil Science, he contributed in the founding of the Agricultural University at Bogor, presently known as IPB (Institut Pertanian Bogor).

Chapter 1

Issue of Organic Matter

1.1 Concept of Organic Matter

1.1.1 Historical Developments of Different Concepts

Since organic matter is the ultimate source and origin of humic matter, it is perhaps necessary to start this book on issues of humic matter with some detailed studies, examining first what organic matter entails and what its relation is to humus and humic matter in the environment. At first recognized only in the *lithosphere*, and particularly in the *pedosphere* as one of the four major soil constituents, today organic matter is also considered in the *hydrosphere* as an important component for the continuation of life. Next to the terrestrial concept of organic matter as represented by *soil organic matter (SOM)*, a new concept has emerged lately to accommodate the issues of organic matter in streams, lakes, and the oceans, called *aquatic organic matter*. It is not exactly a truly new concept but rather an extension of an older idea used in the 1950s or before by limnologists in their studies on biological life and its activity in natural waters. When by the end of the last century, biogeochemists and especially hydrologists at the U.S. Geological Survey became attracted to humic acid research, they also turned their attention to aquatic organic matter and its dissolved fraction that they called DOM. Although aquatic organic matter—assigned here by the author, for convenience, the acronym AOM—is still closely related with SOM, several major differences stand out that make it different from SOM, requiring the two—AOM and SOM—to be treated as separate entities. For example, grass, shrubs, and trees are the major sources of terrestrial organic matter, whereas algae and plankton are the origin of AOM, creating great differences in

quality and chemical composition between the two groups of organic materials. Recognizing AOM as a group of substances, important for biological life and in formation of humic substances in natural waters, has also changed the perception of many other people regarding these substances in water. Regarded by the general public at first as contaminants discoloring stream and lake waters, making them unfit for drinking or other human applications, AOM is now believed by many to be essential for sustaining the health of the ecosystem. In other words, it can be stated that “what was considered dirt is now a valuable material.”

1.1.2 Concept and Definition in the New Millennium

The term *organic matter* seems to invoke a simple straightforward meaning, which is “matter arising from living matter,” and in chemistry and soil science referring more specifically to “matter relating to or containing carbon compounds.” The latter is also the definition presented in *Webster’s New Collegiate Dictionary* (Merriam-Webster, 1973). In basic soil science, it is a general term to describe a mixture of fresh and dead organisms, composed of carbonaceous remains of organisms that once occupied the surface of the earth (Brady and Weil, 2008). This is, in the author’s opinion, what the name organic matter is meant to be. As such, it then qualifies to be called *natural organic matter* or *NOM* (an acronym often used today for quick reference). It is suggested here to apply the name *organic matter* as a collective or umbrella term embracing the complex mixture of a great variety of broken-down materials from all living organisms on planet Earth. More specifically, organic matter is composed of living and dead biomass, mainly of plant origin. The dead fraction is formed by chemical and biological decomposition of live or fresh organic residues and is usually distinguished into the following:

1. Partially decomposed organic residues or organic matter at various degrees of decomposition, in which the morphology of plant material is still visible
2. Completely decomposed materials with no traces of the anatomical structure of the original plant material from which they have been formed

Group no. 1, containing most of the undecomposed material, is often called *litter* when it lies above the ground or on the surface of soils. In forest

and grassland ecosystems, plant litter is not only of particular importance in the process of nutrient cycling, but also has a prominent influence on soil physical properties. It creates a loose and friable soil consistency, lowers bulk density, and increases the water-holding capacity of the soil. This group of organic matter finds practical application in U.S. Soil Taxonomy, where it is distinguished into *fibric* and *hemic* fractions, a distinction based on the relative degree of decomposition. The fibric (Latin *fibra* = fiber) fraction is the least decomposed part, whereas the hemic (Latin *hemi* = half) fraction is the fraction of the organic mixture at a more advanced state of decomposition. The organic matter in group no. 2 is referred to as *sapric* organic matter, coined from the Greek term *sapros*, meaning rotten, and hence is the most highly decomposed organic fraction. Sapric material is commonly dark gray to black (Soil Survey Staff, 1990, 2006). This is the fraction called *soil humus* by many scientists in the old days, and is believed to be the *soil humic matter* (Russell and Russell, 1950; Whitehead and Tinsley, 1963). The two names are used interchangeably, and even today many scientists consider soil humus to be soil humic matter (Stevenson, 1994).

At the current stage of knowledge, the rather confusing perception also persists that in the form as humus, organic matter will remain stable or resistant to any further decomposition, and will change very little physically and chemically overtime. The old article by Whitehead and Tinsley above underscores the idea of humus representing a *quasi-equilibrium stage* in the breakdown of organic residues. Their journal article above is listed in the *Wikipedia* entry on *humus*, as published in 2006, creating a sense of the presence of a very current, modern, citation. However, such a listing rather results in confusing the authenticity of an otherwise good article.* In the author's opinion, the use of the term "quasi-equilibrium stage" can perhaps be justified by reading it as humus being organic matter in a transient stage. It is continuously being decomposed; however, at the same time, it is constantly being regenerated, replacing the older, lost materials. New compounds are also being formed—humic substances—and added to the mixture called humus, changing the perception of humus into one of a rather complex mixture of decomposed nonhumified and humified organic matter. The issues and principles of biomass, organic matter, litter, and the concepts of NOM, SOM, AOM, and DOM in terrestrial and aquatic ecosystems will be examined further in some detail in the following sections below, whereas the concept of humus will be discussed separately in Chapter 2.

* <http://en.wikipedia.org/wiki/Humus>; pp. 1 and 3; accessed September 13, 2011.

1.2 Issue of Biomass

1.2.1 General Concepts and Definitions

The term *biomass* can be defined in several ways, since scientists in biological science seem to formulate definitions to suit their purpose. It is amazing how, in the process, the authorities in the subject have blown up a simple idea “biomass” into a complex and confusing issue. This keeps several of the other scientists also wondering which among the many definitions of biomass is the proper one to use. As it is written, from the two words “bio” and “mass,” it simply means the mass (size, quantity) of biological matter. However, some define it in terms of the number of living organisms or in terms of the weight of living organisms only. Others base their definition of biomass on the number or weight of both living and dead organisms, whereas definitions considering weights of dead organisms only are also found. The units of biomass are generally in tons or kilograms per hectare as mass of wet or dry material. However, units expressed in tons or kilograms of organic carbon are also often used, making the issue only more complicated than it already is. Biomass defined strictly on the basis of the amount or number of one biological species is called *species biomass*. When it is defined on the basis of the total mass of all species together, it is called *community biomass*. The latter is applied by Stevenson (1989, 1994) to formulate his *microbial biomass* definition. The bacterial biomass is estimated to be equal in amount to that of plants. In ecology, a third type of biomass, *global biomass*, is recognized. This is apparently meant to refer to the amount of biomass on a global scale, meaning biomass contributed by the combined actions of tropical rainforests, temperate region forests, marine and other aquatic ecosystems, and more (Groombridge and Jenkins, 2000; Field et al., 1998). For the purpose of the present book, biomass is defined here in terms of the simplest meaning of the name as indicated earlier above. All organic materials derived from plants and animals, alive and dead, are the main sources for the formation of organic matter in soils. In this respect, the author tends to include agricultural and forest crops and their residues as major forms of biomass, although a great part of the crops and trees may be harvested for food and timber production, respectively, thereby preventing most of their residues from being returned to the soil and contributing to the formation of humus and humic substances in soils. Strictly speaking, plant litter also falls within the category of biomass, and hence must be considered as organic matter. It is common in soil science to exclude litter from SOM,

an issue that will be addressed in Section 1.3.1 on terrestrial organic matter in more detail. By being plant remains, litter fits the description of biomass and hence should be included in the definition of NOM. The latter should not be confused with SOM, which scientifically is a lower taxa, a subdivision of NOM, or a *subset*—a name used today by some for a subdivision. The descriptions above explain that biomass may also qualify to be called *biotic matter*.

1.2.2 Concept of Industrial Biomass

Because biomass is rich in energy, it can also function as a renewable source of energy, valuable for the production of biofuel, biodiesel, methane gas (sometimes also called landfill gas or biogas), and ethanol. Palm oil residues and other vegetable oils are the usual material for producing biodiesel, while mention is also made of the importance of eucalyptus plants (Goenadi, 2006; Tan, 2011). Biomass produced specifically by growing plants, such as switchgrass, sugar cane, sorghum, corn, and other plants for industrial purposes as stated above, is called *industrial biomass*, a fourth type of biomass. It is, in addition, also useful in the production of artificial fibers and other chemical compounds. The plain term, biomass, is then reserved by some to refer to biotic matter that is of little significance or not used in biofuel or biogas production. With the recent crisis in fossil fuel production, starting with the 1973 oil embargo, by some of the members of OPEC (Organization of Petroleum Exporting Countries), imposed on the United States and Western Europe, and continuing thereafter because of the seemingly insatiable worldwide oil consumption, the significance of industrial biomass has increased considerably. This has changed the biomass concept accordingly to also define it in terms of an energy source. An example of a definition to this extent is quoted below from the website of the U.S. Department of Interior, Tribal Energy and Environmental Clearing House, Office of Indian Energy and Economic Development: “Biomass is any plant-derived organic matter. Biomass available for energy on a sustainable basis includes herbaceous and woody crops, agricultural food and feed crops, agricultural crop wastes and residues, wood wastes and residues, aquatic plants, and other waste materials, including some municipal wastes. Biomass is a very heterogeneous and chemically complex renewable resource.”*

The biologically derived material, defined above as biomass, was first studied by soil scientists on terrestrial ecosystems; however, late in the 1980s, it has greatly attracted the attention of geochemists, hydrologists, and

* <http://teeic.anl.gov/er/carbon/carboninfo/cycle/index.cfm>; accessed October 29, 2011.

especially hydrobiologists. From their investigations in aquatic ecosystems, it became apparent that wet environments were equally capable as terrestrial sites of biomass production. Depending on the different aquatic environments, several types of aquatic biomass can be recognized, e.g., freshwater, and marine or oceanic biomass. By the end of the twentieth century, the idea surfaces that this aquatic biomass can also play a significant role in humus and humic matter formation in natural waters. This change in perception and the major differences noted with respect to origin and resulting composition are reasons for the present author to suggest a division of biomass into two broad groups, i.e., terrestrial and aquatic biomass. The two groups are compared below in more detail.

1.2.3 Terrestrial Biomass

The term *terrestrial*, coined from *terrestris* (Latin for *Earth* or belonging to Earth), is commonly used to refer to environments on “dry” land or the lithosphere and pedosphere. Hence, *terrestrial biomass* can be defined as the mass of organic matter originating mostly from trees, shrubs, and grass, generally from all plants growing and dying on land areas, soil, or terrestrial ecosystems. Roots and microbial organisms are significant contributors to terrestrial biomass formation, whereas insects and higher animals are adding some or little to this. The foregoing can be restated in scientific jargon as follows: “terrestrial biomass decreases significantly from lower to higher trophic levels of the food chain, meaning that amount of biomass is higher in plant materials than in insects and herbivores, with the carnivores producing the lowest amounts.” Therefore, the highest predators or organisms at the highest trophic levels are contributing the smallest amounts to biomass. For example, the biomass of a tropical region forest is estimated to amount to 500–600 tons/ha, 300–400 tons/ha in a temperate region deciduous (broad leaf) forest, as compared with the 20–50 kg/ha produced by vertebrates. Earthworms were reported to produce approximately 1000 kg/ha (Purvis and Orians, 2007; Foley et al., 2007). For this reason, the concept of biomass in soil science is formulated only on the basis of plant materials. If higher animals are to be counted too, hundreds, if not thousands of them, should be found littering the land surfaces. Even during a catastrophic disaster, such as with the extinction of dinosaurs, this is not likely to occur. The concept of *trophism* and *trophic states* in relation with biomass production will be addressed further in some more detail in Section 1.5.1.

1.2.4 Aquatic Biomass

Aquatic biomass is a more complex issue than the terrestrial type discussed above. Aquatic environments are ecosystems in bodies of water or the hydrosphere. They can be distinguished into *marine* and *freshwater ecosystems*, each exhibiting different zones populated by different organisms as potential contributors to the formation of aquatic biomass. Marine ecosystems recognize oceanic, profundal, benthic, intertidal, estuaries, salt marshes, coral reefs, and hydrothermal vents. Notwithstanding such a diversity in zones or habitats, the most important sources for marine biomass formation are plankton, kelp, and brown algae. Krill, which is classified as an animal species, is also considered a highly significant biomass contributor in arctic marine regions. The higher *trophic level* of organisms, e.g., sharks and other predatory fish in oceanic zones, is of less importance for the production of aquatic biomass. Freshwater ecosystems, on the other hand, are less complex by recognizing only *lentic* (standing water bodies), such as ponds and lakes; *lotic* (moving water in streams); and wetland systems. The lentic ecosystems, in general, are ecosystems enabling a great variety of aquatic weeds to flourish, such as *Salvinia* (*Pteridophyta* sp.), water hyacinth (*Eichornia crassipes*), *Hydrilla* sp., and *Lemna* sp. These are to name just a few examples important as major contributors to freshwater biomass formation. Blue-green algae are also recognized as a significant source for its formation. A lake ecosystem, specifically, recognizes a (1) *pelagic* zone, which constitute an open offshore water body; (2) *littoral* zone for near shore waters; and (3) *riparian* zone, which is the land area bordering the lake. This is the area of lakes most affected by accumulation of biomass. In general, the biomass alongside moving streams is derived mostly from trees growing in the riparian forest, whereas that in large slowly moving rivers and lakes is derived mainly from algae and aquatic weeds as indicated earlier above. Over the years, the lentic systems of lakes and ponds tend to become enriched with plant nutrients, resulting in a phenomenon called *eutrophication*. The process causes an excessive growth of aquatic plants, including the development of the dreaded *algal bloom* (Tan, 2009). Nevertheless, in terms of freshwater biomass, the process of eutrophication above is beneficial for the formation and accumulation of biomass. Blue-green algae are also known to be rich in energy and thus are also categorized as industrial biomass. Their potential for use in the production of biofuel has attracted lots of attention since 2003.

1.3 Terrestrial and Aquatic Organic Matter

In its development into a modern science, the concept of organic matter becomes far more complex, and often also more confusing than expected. As indicated before, it was first studied by soil scientists as organic matter on land areas or soil ecosystems. However, at the end of the twentieth century, it also attracted considerable attention from hydrologists, hydrobiologists, and geochemists, who tried to underscore the great role organic matter also has in aquatic environments. For a long time, organic matter in aquatic ecosystems was studied at first from the perspective of its importance in limnology and hydrobiology. The change in perception as addressed earlier and the rapid development in chemical analysis and improved isolation techniques by gel filtration have contributed, close to the turn of the century, to a sudden surge in the science of AOM and its close relative—the “aquatic humic substances.” A number of investigations were initiated in 1965 by scientists in Norway (Gjessing, 1965), followed by more recent research in the 1980s by the late Ronald Malcolm and coworkers—founders of the International Humic Substances Society (IHSS)—at the U.S. Geological Survey, Denver, Colorado (Aiken et al., 1985). Since then, a number of articles were reported from other countries (Steinberg, 2003; Gjessing and Källqvist, 1991; Gjessing et al., 1999). As a result of all these activities, new ideas were suggested, several fractions of dissolved organic matter (DOM) were allegedly discovered, and allochthonous and autochthonous issues surfaced, and more. In the author’s opinion, the presence of this type of organic matter and in particular its dissolved fraction, earlier called DOM, is not limited to natural waters only, but DOM is also available in terrestrial soil ecosystems. The soil contains water as one of its four major constituents, which is known as *soil water*, *soil moisture*, or *soil solution* (Tan, 2009). Depending on how wet or moist the soil is, this water is present in the pore spaces, as interstitial water bridging solid particles, as interlayer water, and as hydration shells and films around solutes and colloidal particles. These are the most likely places in soils where DOM can be found in varying concentrations as the decomposition and weathering products of SOM. Therefore, underscoring the great importance of DOM for both terrestrial and aquatic systems, the author believes that it may assist in providing the key toward solving the nature and characteristics of humic substances. More details on specific issues of terrestrial and aquatic organic matter and their DOM fraction are provided in the following sections.

1.3.1 Terrestrial Organic Matter

Terrestrial organic matter can be present in many forms, and as the term *terrestrial* implies, it is developed on terrestrial—dry land—ecosystems. It includes, therefore, not only organic matter in the soil, derived from plant biomass, but also organic matter in the form of geologic deposits, such as lignite, leonardite, kerogen, bitumen, and coal. These materials are products transformed from terrestrial organic matter by a process called *metamorphism* over geologic ages. For example, kerogen is found, often in substantial amounts, as the organic fraction of sedimentary and metamorphic rocks. On the other hand, lignite or leonardite is discovered as geologic deposits and is useful in industry as a source for the commercial production of humic acids. All of them are in fact known not only to be rich in humic matter but also constitute very important sources for the production of natural gas and oil. At the proper elevated pressures and temperatures in the earth's crust, they are believed capable to be transformed into crude oil or natural gas by metamorphic forces—referred to as *geothermal pressures* by geologists and geochemists—acting in nature (Tan, 2009). Peat deposits also qualify to be categorized as terrestrial organic matter, although peat starts at first to form in wetlands. These lands are often considered creating aquatic ecosystems because of their wet conditions for some or most part of the year.

1.3.1.1 Soil Organic Matter or SOM

In a narrow sense, most soil scientists use the name *soil organic matter*, identified today often as *SOM*, for the organic materials in “dry” land ecosystems, instead of the term *terrestrial organic matter*, which has a wider meaning. The acronym SOM is, in fact, used by some for all organic matter present in soils, excluding charcoal (Oades, 1988), and/or for all organic matter occurring in soils, excluding nondecayed plant materials, their partial decomposition products, and the living biomass (MacCarthy et al., 1990). Baldock and Broos (2011) define SOM as “the sum of all natural and thermally altered biologically derived organic material found in the soil or on the soil surface, irrespective of its source, whether it is living or dead or stage of decomposition, but excluding the aboveground portion of living plants.” Although the latter is a nice definition, excluding the live aboveground parts only may raise questions about whether *live plant roots* are meant to be considered as SOM. Regardless of the variations noticed in the definitions above, all the quoted definitions tend to underscore organic matter existing in the

soils' systems only and not that occurring in terrestrial systems. Apparently, often, it also depends somewhat on the clout of the authority in organic matter which one of the several definitions published in the literature will be preferred and accepted by the general public. The author will leave it up to the reader which one to choose.

Fresh and dead plant materials and partly decomposed residues, collectively called "organic matter" above, tend to be incorporated thoroughly into the soil by live biotic activities and abiotic processes such as weathering and leaching. Making up the *soil organic fraction*, it is one of the four major groups of soil constituents (Brady and Weil, 2008; Tan, 2011). Together with the other three groups of soil constituents—mineral matter, water, and air—the incorporated organic fraction is building up the *soil*, or more scientifically, the *pedon*. The *pedological concept* in soil genesis assumes soils to come into existence only after organic matter has been established in the "inorganic mass" derived from weathering and decomposition of rock debris. However, dead plant material, known as litter, is not considered as soil organic matter or SOM in U.S. Soil Taxonomy, but recognized as part of a soil profile or soil horizon and assigned the symbol "O" (for organic). This O horizon, defined similarly as plant litter containing undecomposed or partially decomposed litter, such as leaves, needles, twigs, moss, and lichens deposited on the surface or top of either mineral or organic soil (Soil Survey Staff, 1990, pp. 413–414), can be divided into the O, Oi, Oe, and Oa subhorizons. The O subhorizon is the top horizon composed of the fresh plant residues, whereas the Oi, the next subhorizon underneath, defined as a slightly decomposed layer, is the fibric subhorizon. The following underlying layer is the Oe subhorizon containing intermediately decomposed organic material, earlier called hemic matter. The bottom part directly in contact with the mineral soil surface is then the Oa subhorizon, which, by definition, possesses the highly decomposed part of the litter layer, previously called sapric matter.

1.3.1.2 Issue of SOMM

Late in the 1900s when modeling in soil chemistry and other scientific aspects had attracted great attention, biologists at St. Petersburg Forest Technical Academy, Russia, tried to develop a model of the processes of formation of SOM that they called SOMM—Soil Organic Matter Model or Modeling (Chertov and Komarov, 1997). It is apparently an attempt to come up with a quantitative concept, reflecting the processes of SOM

formation—called soil organic matter dynamics by the authors—from litter fall, to mineralization and transformation or synthesis into humic substances, to bonding by clay minerals, as illustrated by the flow-sheet provided for the SOMM model. It is, in essence, only a name for referring to a series of linear differential equations with six variable coefficients, depicting the alleged rate or degree of mineralization, synthesis into humic substances, and other decomposition reactions involved. Each of the coefficients, assigned the symbols k_1 through k_6 , is formulated independently as a linear regression equation. Because of the complexity of the statistics, the readers are referred to the journal article of Chertov and Komarov (1997). The authors have applied the concept of quantitative modeling for the formation of mor, moder, and mull, the major humus types in forest soils. In their desire to include the different views in pedology, biology, ecology, and silviculture, the research was instituted as a cooperative study with crops and soil institutions in Australia; Czech Republic; Germany; Rothamsted Experimental Station, UK; and Duke University, Durham, North Carolina, as obvious from their report (Chertov and Komarov, 1997). The data from three plots at the Rothamsted Park Grass fields in the United Kingdom, where hay was cut twice annually since 1856, showed carbon (in tons/ha) plotted against the year. The resulting linear regression curves were almost invariably horizontal, suggesting a model with very little changes in formation and accumulation of “humus” from 1875 to 1985 or to 1990. Similar results were reported for the plots in the different countries. The lack of statistics reflecting degree of significant differences, e.g., LSD_5 (least significant difference at the 95% level of probability), σ = standard error, r = regression coefficient or r^2 , and/or the like, makes it very difficult to comment on the reliability of results. The concept is also very difficult, if not cumbersome, to apply in normal research investigations without access to experimental fields programmed for long-term studies, whereas the excessively large amounts of data to be collected require the availability of reliable computer programs.

1.3.1.3 Litter–SOM Connection

Although, strictly speaking, plant litter is the primary and only source for the production of SOM, the consensus is to exclude litter from the definition of SOM, as indicated above. Accumulated on top of the soil, plant litter is often coarse and too large in size to be considered a soil constituent. Soil scientists would not consider a layer of pine needles or a dead twig or tree limb on the ground as a soil constituent. The issue is perhaps in analogy

to rocks, stone, and gravel, which in pedology are also not called soil constituents because of their large sizes and unweathered conditions. However, as a result of weathering and decomposition, they are producing sand, silt, and clay, the mineral matter of soils. The clay is the colloidal fraction of this mineral part of soil particles produced by weathering, similarly as humus is the colloidal part of the soil organic fraction created by decomposition of plant litter. Both the soil's clay and humus are the chemically active parts of the soil system. Regardless of the opinion above in excluding litter as SOM, the present author wonders about the possibility to include the bottom part of a litter layer, in direct contact with the soil surface, within the definition of SOM for the following reasons. It is continuously broken down or decomposed to such an extent that it is transported into the soil and mixed with other soil particles by the combined actions of leaching and of burrowing animals, insects, and microorganisms, called *detritivores*. Earthworms are good examples of detritivores, and so are millipedes, slugs, and other insects, although their actions in the decomposition process may differ from the earthworms. As heterotrophic organisms, they all obtain their nutrients by consuming organic debris, resulting in most of the original plant materials becoming smaller in size and eventually transforming into a combination of fine organic particles. The new product, in which the original plant material has become nonrecognizable to the eye, is called *detritus*. In terrestrial ecosystems, detritus is deposited on and in the surface soil, creating a dark-colored humus-rich layer of surface soil—the soil's A horizon—practically directly beneath the litter layer of fresh dead leaves and twigs. Some scientists consider detritus as similar to humus, which is in fact correct considering its definition of being a nonrecognizable mass of decomposed organic mixture. In aquatic environments, detritus is often present as a suspension, which eventually may settle down toward the bottom of streams and lakes.

1.3.1.4 Importance of SOM

As explained above, SOM, derived from the soil biomass, is composed, strictly speaking, of both living and dead organic matter. However, in dealing with SOM issues, people are most often accustomed to referring only to the dead fraction, ignoring its living fraction for reasons unknown to the author. The organic matter content of soils is usually substantially smaller than the soil's clay content. For example, organic matter contents are only 2% to 4% ($= \pm 1\text{--}2\% C_{org}$) in Oxisols and Ultisols, soils known to possess

high clay contents varying from 80% to 40%. In Mollisols and Andisols, considered organic matter-rich soils, the clay contents are usually in the range of 30% to 40%. However, their SOM contents are only 6% to 12% ($\pm 3\text{--}6\%$ C_{org}) (Stevenson, 1994; Soil Survey Staff, 2006; Brady and Weil, 2008; Tan, 2009). Nonetheless, the soil organic fraction, although much smaller in amount, is the most important fraction in soils. It is recognized today that their chemical activities far exceed that of the soil mineral fraction, in particular exceeding that of the colloidal clays. Soil organic matter has attracted considerable attention since the early days of agriculture because of its pronounced effect on the physical, chemical, and biological condition of soils (Russell and Russell, 1950; Tan, 2011). It is noted today to increase the soil's capacity for adsorption and retention of water more than the soil's clay fraction. It is renowned for improving soil structure; providing a loose, friable soil condition; lowering bulk density values; and increasing the soil's cation exchange capacity. Soil organic matter is the main source of nitrogen (N₂)—an essential nutrient for plant growth—in the soil, and more. The growth and yield of crops have always been noted to be better when plants are grown in soils rich in organic matter. Lack of scientific data and difficulties in separating and/or isolating it in the past from the soil mineral fraction are perhaps some of the reasons for the presence of reservations as to its beneficial effect on soils and plants (Russell and Russell, 1950; Stevenson, 1994).

1.3.2 Extraterrestrial Organic Matter

Mention is made today of the presence of *extraterrestrial organic matter*, as if to make things more complicated. It is organic matter not formed on Earth, but produced in the solar system (Latin *extra* = “not from” and *terrestris* = earth). This idea seems to originate from speculations about the existence of extraterrestrial life, the science called today *astrobiology* or *exobiology* (Mix, 2005). If life exists in our galaxy in the form of bacteria to organisms far advanced than human beings, some type of organic matter then should exist related to these hypothetical extraterrestrial living things. NASA (National Aeronautics and Space Administration) scientists are also convinced of the possibility of organic matter production in our solar system (NASA Astrobiology Institute, 2011).

This existence of extraterrestrial organic matter has attracted increasingly greater attention lately because of the recent discoveries of organic carbon

in interplanetary dust particles or IDPs, also known as *cosmic dust*, and in meteorites (Irvine, 1998; Bhardwaj, 2007). For example, the *Murchison* meteorite—a meteorite that hit the earth on September 28, 1969, near the town Murchison in Victoria, Australia—was reported to contain carbonaceous chondrites. Kerogen was discovered in chondrite meteorites and interstellar dust. At the current state of knowledge, the present author has no experience commenting on what the effect is of extraterrestrial organic matter on the formation of humus and humic matter in space and on Earth. Whether kerogen can also be a source for the production of fossil fuel in our galaxy is a matter of speculation. The topic of extraterrestrial organic matter is added only for completeness, with the intention to stir interest from other scientists and professionals. At the present time, the idea of astrobiology on the nature of extraterrestrial organic matter is, in principle, only focusing on the presence of a variety of amino acids, purines, pyrimidines, and other organic chemicals with a perception somewhat different from that held by soil scientists about SOM.

1.3.3 Aquatic Organic Matter

Today a lot of attention has been directed toward the study of AOM by hydrologists, hydrobiologists, and biogeochemists. As indicated before, at first an area of research in limnology, it was later expanded by examining the safety of natural waters for drinking and other domestic applications. U.S. Environmental Protection Agency (U.S. EPA) personnel were in the late 1970s concerned about DOM playing a key role in the production of harmful by-products as a result of treatment of natural drinking water supplies with chlorinated disinfectants (Ryan, 2010; Aiken et al., 2011). Since then, more people have become involved in the study of AOM (Gjessing et al., 1999). In the period when interest in humic substances was soaring to an all-time high among U.S. Geological Survey scientists following the foundation of the IHSS in 1985, the investigations were directed more toward issues related to humic matter in natural waters.

1.3.3.1 Concept of AOM and DOM

Simply defined, AOM is organic matter present in aquatic environments; however, in the true context of its scientific definition, it is an integrated mixture of humic substances in natural waters composed of broken-down, partially and totally decomposed remains of plant biomass, as clearly stated

in Section 1.1, on the concept of organic matter. The concentration of the humic substances is usually substantially higher than the nonhumified material (Thomas, 1997; Bertilson and Tranvik, 2000). However, the name DOM seems to be used invariably for referring to this mixture of AOM as a whole in a great number of articles. The terms DOM and AOM are applied interchangeably, as if there were no principal differences between them, resulting in confusion for interested people who are not in hydrology or geology. As the term DOM implies, it is the soluble fraction of organic matter, and as such it is only part of what was defined earlier as SOM, and in particular part of the humus fraction. An additional controversy is the use of the name DOC (dissolved organic carbon) to replace DOM. It is of course natural to relate organic carbon to organic matter; however, in the author's opinion, DOM and DOC are two different parameters with different perceptions. As the names imply, DOM refers to *dissolved organic matter* as a whole, whereas DOC refers to dissolved organic *carbon*, the elemental constituent that makes up organic substances. It is common in analytical chemistry or soil analysis to consider organic matter to be composed, on average, of 57% organic carbon (Jackson, 1960; Tan, 2005). This figure, converted to a factor of 1.7, is then used in the calculation for conversion from C_{org} to organic matter. Hence, when talking about DOM, one is referring to almost twice the amount of organic matter than that expressed in terms of carbon or DOC.

1.3.3.2 Origin of AOM and DOM

Although AOM, as a whole, is expected to originate from the aquatic biomass and its remains (see Section 1.2.4), the author believes that the terrestrial biomass and its remnants may also have greatly affected its composition, especially near shorelines of freshwater lakes and in intertidal zones and estuaries of marine ecosystems. Originating in riparian zones, the remains of the terrestrial biomass were transported by erosion, runoff, leaching, percolations, and sometimes floods from higher grounds into the lakes, ponds, streams, estuaries, and intertidal areas, where they became an integral part of the AOM system. The organic substances of terrestrial origin are here referred to as *allochthonous* organic matter, in contrast to the other part, known as *autochthonous* organic matter. The autochthonous fraction has been derived from the indigenous aquatic organisms, such as algae, phytoplankton, kelp, and krill. Surrounded completely by water during their growth, the indigenous aquatic plants do not need lignin in their systems. The composition of autochthonous organic matter is therefore more

likely less lignoid and phenolic in character than the allochthonous fraction, a topic that will be discussed in more detail in the section on the issue of DOM below. Both allochthonous and autochthonous AOM are composed of *solid* and *dissolved organic matter* or *DOM*. The solid part is not dissolved in water, but is present as a suspension, while some of it may be in colloidal forms and hence do not qualify to be called DOM, as identified in most journal articles (Aiken et al., 2011; Dubnick et al., 2010; Leenheer and Croué, 2003).

The contribution of the allochthonous fraction in the formation of AOM allegedly exceeds that of the autochthonous fraction as has been studied in Finland by Tulonen (2004) in large humic lake ecosystems. The term *humic lakes* apparently refers to lakes containing black water. Reference is also made by the author regarding the importance of this organic mixture as a DOM pool for utilization by heterotrophic bacteria. Algae and phytoplankton are traditionally considered the main sources for the production of autochthonous DOM. However, macrophytes, such as salvinia, water hyacinths as mentioned earlier, mosses, and other vascular plants rooted in the substrates of streams and lakes have lately received much attention as they are also important autochthonous sources of DOM in natural waters, especially in littoral zones of the smaller lake ecosystems (Finlay and Sinsabaugh, 2002).

1.4 Issue of Dissolved Organic Matter or DOM

1.4.1 Operational Concept of DOM

Dissolved organic matter or DOM is an *operationally defined* organic fraction and, until recently, this perception is used in analogy with the operational concept of humic substances of the 1980s (Leenheer and Croué, 2003; Aiken et al., 1985). However, since the turn of the century, humic substances are now recognized as definite substances occurring in nature. Their concept as *operational compounds* became obsolete by the end of the twentieth century (Tan, 2011; Piccolo, 2002; Wershaw, 1999). On the other hand, the definition of DOM may still change according to the method used for isolation or separation and its classification, as DOM shows a wide variation from author to author. Gjessing et al. (1999) call it NOM, as do Wershaw et al. (2005), who also use the name NOM instead of DOM for aquatic organic matter in a water reservoir system of New York. In soil science, DOM compounds are considered, on the one hand, components for the formation of

aquatic humic substances, and, on the other hand, substances released from the degradation of these types of humic acids. In the early efforts of isolating aquatic humic substances by the XAD-8 method, materials retained by the resin are defined as humic substances, whereas all other materials flowing through are called DOM. This method was first presented by Thurman and Malcolm (1981) and later adopted as the IHSS procedure for the separation of humic from nonhumic matter—now called DOM—in black water (Aiken, 1985). Using a revised isolation method composed of a serial two-column array of XAD resins, the isolated nonhumified fraction was later called by U.S. Geological Survey hydrologists *transphilic DOC* (Leenheer and Croué, 2003; Wershaw et al., 2005), making the issue of DOM more confusing than it already is.

Notwithstanding the issue of DOM being defined as operational substances, the fact is that AOM exists in nature and DOM is part of it. The yellowish-brown, brown to black colors of many streams and lakes in Northern Europe, Africa, Asia, and North and South America signify its presence in nature.

The Rio Negro, an Amazon River tributary, in Brazil, and many streams and swamps in the southeastern United States carry dark-colored water attributed to AOM. Streams draining huge peat lands in Southeast Asia and other parts of the world are generally dark in color (Tan, 2008). Called *black water* by the present author, its relation with humic matter will be discussed in more detail in Section 2.2. In the present section, it is examined only to provide additional background information and at the same time to shed some light as to the great importance it has attracted from geological scientists.

1.4.2 Pore Size Limits and Molecular Weights of DOM

Today, DOM is defined by a number of hydrologists or hydrobiologists as that part of AOM passing through 0.2–0.8 µm or 0.45 µm pore filters and the like. On the other hand, marine scientists define DOM as organic material that flows through a GF/F fiberglass filter (Aiken et al., 2011; Dubnick et al., 2010). However, scientists at various institutions in Australia, Italy, New Zealand, United Kingdom, and United States define DOM as organic fraction that passes through a 0.45-µm filter (Bolan et al., 2011). The above procedures are reported to be also the methods for isolation of DOC, adding to the controversies in the use of the terms DOC or DOM (see http://en.wikipedia.org/wiki/Dissolved_organic_carbon; accessed December 28, 2011). One cannot help wonder how organic carbon—as the name implies, C_{org} (an element)—can exist in solution and be isolated by filtering as such.

Most agree that the filtered DOM obtained represents only a small fraction of the total organic matter present in natural waters. Aquatic humic matter makes up the bulk of the latter. The DOM obtained is also not uniform in size and structure, as noted by ecologists at the University of Braunschweig, Germany. They indicate that their DOM isolated with a 0.45- μm filter is composed of materials from 0.45 μm in size down to smaller molecules present as a continuum (Nieder et al., 2003). By using a membrane filter with a similar 0.45 μm pore size limit, followed by Amberlite XAD resin separation, Leenheer and Croué (2003) claim to even have managed to separate DOM into the following four fractions: (1) colloidal, (2) hydrophobic, (3) transphilic, and (4) hydrophilic organic fractions. However, no analytical details are provided, but only a flow sheet is presented. Some of the analytical procedures were presented later by Wershaw et al. (2005). In view of the upper size limit of soluble substances set at 0.001 μm by Aiken et al. (2011), the colloidal fraction above is then insoluble in water and does not qualify to be listed as DOM. However, setting sharp limits, at which colloidal substances become soluble or insoluble, is arbitrary because the size of colloids represents a continuum, an issue examined in detail by Tan (2011). The suggestion in this respect, as proposed by Wershaw (2004), to divide DOM in natural waters into (1) truly dissolved DOM and (2) colloidal DOM, does not solve the solubility issue of colloidal materials. Perhaps it is better to list the colloidal fraction obtained by Leenheer and Croué (2003) at the same level as DOM; in other words, as part or as a subdivision of AOM as a whole.

The DOM obtained is reported to exhibit molecular weights ranging from a few hundred to 100,000 Da (daltons). Unfortunately, none of these molecular weights can be used to characterize DOM, and any number within that wide range can be assigned to DOM. The high molecular weight fraction is called RDOM (refractory DOM) by Tulonen (2004) because of its refractory nature upon analysis. Humic matter is included in this fraction. The low molecular weight components are grouped together as biologically labile DOM, or LDOM.

1.4.3 Composition of DOM

Generally, DOM is believed to be composed of a complex mixture of aliphatic and aromatic carbon compounds whose functional groups may contain attached amino acid, amide, and ketone components. Dissolved organic matter, or DOM, highly affected by allochthonous matter, is noted to be low

in nitrogen content but contains relatively high amounts of aromatic and phenolic components, owing to the lignin content by origin from the terrestrial biomass as indicated earlier. On the other hand, the autochthonous DOM is relatively higher in nitrogen but lower in aromatics and phenolic components because of the low lignin content in its original materials. It is assumed to be derived from microbial decomposition of the indigenous aquatic biomass, such as algae and phytoplankton (Tulonen, 2004; Aiken et al., 2011). Algae, in particular, are low in lignin and considered an important producer of autochthonous organic matter because of their capacity to transform solar energy into reduced carbon substances. In the oceans and large lake waters, phytoplankton is considered the main source for this type of AOM. After cell death, the photosynthates are released into the surrounding water as dissolved materials or in the form of particulate detritus. The major soluble compounds contributing to DOM are noted to be proteins, polymeric sugars, lipids, and various monomeric compounds of molecular weights smaller than 600 Da (Bertilson and Jones, 2002). Extracellular release of soluble compounds contributing to DOM has been reported to also occur through leakage across cell membranes and by active secretion or excretion by the algae. These are, according to the authors above, composed mainly of glucose, arabinose and other monomeric sugars, carboxylic acids, amino acids, some glycerols, and mannitols. Both in the body of natural waters as well in the sediments, bacteria are the major organisms capable of decomposing the organic matter in the AOM pool. In doing so, they are not only producing bacterial biomass but these organisms are also inducing mineralization of the soluble and particulate organic components, a process known in terrestrial environments to contribute to nutrient cycling. Tulonen (2004) also reported that in the process, part of the insoluble substances is converted into DOM materials. Bacterioplankton is mentioned to be effective in the mineralization process above, significantly affecting the carbon flow in aquatic ecosystems. These bacteria are also considered a major food source for organisms such as protozoa.

1.5 Effect of Organic Matter on Ecology and Hydrochemistry

From the preceding discussions, it is perhaps clear by now that two major groups of organic matter can be distinguished. The first group is the terrestrial organic matter, represented by SOM and geologic deposits of organic

matter, such as lignite and kerogen as addressed earlier. Soil organic matter is of importance in plant growth and agricultural operations, whereas lignite, kerogen, and the like find application in industry as sources for the production of commercial humic substances and/or oil. The effect of SOM on the physical, chemical, and biological properties of soils, improving crop production, has been reported in many soil textbooks (Brady and Weil, 2008) and are also standard topics for teaching basic soils at universities worldwide. To avoid being repetitious, the effect of terrestrial organic matter will now focus more on the other less reported but more related topics of the current book, e.g., ecology and humic substances. The second major group of organic matter is AOM, represented by DOM, which has been promoted by hydrologists and/or biohydrologists as equally, if not more, important in the study of humic substances as SOM. Its effects in water can perhaps be summed up as follows (Wright and Reddy, 2009; Tulonen, 2004; Nieder et al., 2003; Thomas, 1997; Stephens, 1968). As indicated earlier, the dissolved fraction or DOM comprises only a small part of the total mass of AOM; yet, in the form of dissolved material, it is the most mobile organic fraction. Therefore, DOM is more likely to affect easily a great number of reactions occurring in the aquatic environments. Its presence affects the taste, odor, and color of natural waters, and as indicated in Section 1.3.3, it is also believed to produce undesirable disinfection by-products, making water unfit for drinking or other domestic application. Claims abound in the literature of DOM functioning as a carbon sink, and as such may have an important role in the global carbon cycle. Bolan et al. (2011) indicate that through microbial respiration, DOM in terrestrial and aquatic ecosystems will be broken down into CO₂, which being a gas is returned to the air, thereby starting again the carbon cycle. Dissolved organic matter is also reported to serve as important carbon and energy sources for aquatic biota, which need oxygen and nutrients for living and performing biochemical reactions. These processes may affect the dissolved oxygen content and soluble nutrient concentrations, e.g., N, P, S, and trace elements. In addition, an assortment of organic acids, including humic substances, is produced by the decomposition of DOM, changing the chemistry of water by proton transfer. Some of the compounds produced may also exhibit unique properties, enabling them to behave as Lewis acids, of importance in complexation reactions. By forming complexes and chelates with many chemicals, DOM and its decomposition products may affect mobilization and immobilization of organic and inorganic contaminants. In addition, formation of metal complexes or chelates enhances metal solubility on the one hand, while also reducing bioavailability on the

other. Recently, it has been noticed that AOM is also capable of mediating photochemical reactions transforming DOM materials into DIC (dissolved inorganic carbon). Most of the effects of AOM summarized above are not much different from those of terrestrial organic matter and can be found easily in basic soils textbooks. Therefore, only some selected issues that are perhaps not considered “everyday” topics and deserve more attention will be addressed below in more detail.

1.5.1 Effect on Ecology

Aside from the physical, chemical, and biological effects, the issue of organic matter serving as a pool of food and energy for organisms in terrestrial ecosystems is seldom addressed and underscored as an important process as is the case with AOM. Especially the dissolved fraction, called DOM above, was considered an important pool of organic matter providing food and energy for aquatic organisms at the lowest trophic levels. Such a trophic concept should even be of greater significance in terrestrial ecosystems where organisms, capable of producing food through capturing solar energy by photosynthesis, are more abundant than in stream and lake waters. These terrestrial organisms, usually placed at the bottom of the food chain, are called *producers* and their presence may in turn serve as food for the development of organisms at subsequent higher trophic levels, called *consumers*. The word *troph* (Greek) means feeding, and *trophism* or feeding relation among living organisms is then one of the disciplines of *ecology* or the *study of life* (<http://en.wikipedia.org/wiki/Ecology>, accessed December 17, 2011). In the *Webster's New Collegiate Dictionary*, ecology is defined as “the relationship of living organisms with respect to each other and their environment” (Merriam-Webster, 1973). A *trophic level*, composed of a group of organisms, is then defined as the level they occupy in the food chain. The lowest trophic level, at the base of the food chain, consists of autotrophic organisms capable of producing their own food by photosynthesis, as indicated above. These organisms, earlier called *primary producers*, obtain their nutrients for their growth from elements that are released by decomposition of organic matter by microorganisms, known as *decomposers*, e.g., bacteria. The process is what soil scientists call *nutrient cycling*. The next trophic level, stacked above, is then composed of organisms, called *primary consumers*, that feed on the producers and their photosynthates. In the presence of secondary and tertiary consumers, stacking of trophic levels continued to the apex to form a diagram known as the *ecological pyramid* in ecosystems. In

the author's opinion, two types of ecological pyramidal concepts are apparently available, one that applies to terrestrial ecosystems and another one for use in aquatic and marine environments. Although the two are closely related, the basic components for defining the trophic state are based on different organic species.

1.5.1.1 Terrestrial Ecosystems

Trees, shrubs, and grasses are the *primary producers* in terrestrial ecosystems and are placed at the base of the food chain, hence are frequently called organisms at the lowest trophic level. They contain the highest amounts of biomass, which serve as food and energy for herbivores, known as the *primary consumers*. The latter, in turn, will be consumed by the carnivores, which are then called *secondary consumers*. This is the reason for stating that biomass generally decreases from lower toward subsequent higher trophic levels. The ecological impact of all the above can perhaps be summarized simply as follows. Adequate supply of terrestrial or soil organic matter is known to improve the physical, chemical, and biological conditions of the soil, hence improving the growth medium for trees, shrubs, and grass. The resulting luxurious or better vegetative growth is expected to affect beneficially the life of herbivores, which in turn has a favorable cascading effect on the population of carnivores as the secondary consumers. The latter will in turn also beneficially affect the *tertiary consumers*, if any. In other words, the adequate supply of SOM may create an overall healthy, balanced ecosystem. On the other hand, vegetative growth is usually poor in soils with low organic matter contents. The amount of biomass produced by the primary producers is then inadequate to sustain the life of herbivores. Lack of adequate numbers or amounts of the latter may harmfully affect the life of the secondary consumers, the carnivores, and so on. Improving the deteriorated ecosystems can be achieved by the application of organic matter in the form of compost, stable manure or green manure, and the like, a process commonly carried out in organic farming.

1.5.1.2 Aquatic Ecosystems

The relation between biomass and trophic levels is apparently based on a different organic species in aquatic systems as compared with terrestrial environments discussed above. The U.S. EPA uses the algal biomass to define the trophic status in aquatic conditions (Carlson, 1977).

Algae—phytoplankton in marine ecosystems—are at the bottom of the food chain. Considered as the primary producers capable of producing food by photosynthesis, algae are ranked as organisms at the lowest trophic level. They also get the essential nutrients for growth from those released upon mineralization of AOM by the decomposers, mainly bacteria. Bacterioplankton has earlier been reported to be capable of breaking down allochthonous as well as autochthonous organic substances (Tulonen, 2004) by a process called nutrient cycling. The algae serve as food for small fish, the primary consumers, which in turn are consumed by bigger fish and the latter by larger predatory fish, and so on. In marine ecosystems, otters and sharks are ranked as organisms at the top of the food chain. Summarizing, it can then be concluded that plankton and algae are at the lowest trophic level and this level increases up the trophic tier from fish toward otters and sharks. In general, living organisms at the top of the food chain, ranked at the highest trophic levels, are contributing less to the amount of biomass.

Since AOM, and especially DOM, are considered major carbon sources for algae, phytoplankton, and zooplankton (Tulonen, 2004), their adequate supply in natural waters is therefore important in enhancing the growth of the primary producers. The increased population of the latter, in turn, may beneficially affect the development of aquatic organisms at subsequent higher trophic levels, such as fish, ensuring in this way the development of a biologically healthy aquatic ecosystem. In natural conditions, an adequate supply of AOM may, therefore, transform oligotrophic lakes (lakes with low algal production) into mesotrophic (meso = intermediate) lakes, which are lakes containing medium to high levels of algae, just to the point for creating conditions that are considered biologically productive. Such environments are capable of supporting large amounts of plants and a great variety of fish without the choking effect of algal bloom in eutrophic and especially hyper-eutrophic (hyper = excessive) environments. With an oversupply of nutrients originating from inorganic fertilizers by runoff from surrounding agricultural lands and residential sewage, a hyper algal growth is created because of eutrophication of stream and lake waters. This will consume all the dissolved oxygen, which results in suffocating especially fish upon many other things. For the basics and principles of eutrophication and oligotrophication, reference is made to Tan (2009).

The principles of mesotrophication form the basis of aquaculture in the United States as formulated by H.S. Swingle and coworkers, at the Agricultural Experiment Station, University of Alabama. To raise bluegills

and bass, freshwater ponds are fertilized with N, P, and K to an optimum NPK–fish ratio (Shell, 1975). Such a practice has spread since then to other countries and especially to Thailand, where ponds and lakes are seeded artificially with *Spirulina*, an algae species, in commercial fish farming (Tan, 2009). In Asian countries, organic matter is often applied to stimulate algae growth in ponds and lakes. Such a practice then qualifies to be called *organic fish farming* or *organic aquaculture*. On the other hand, allowing AOM to accumulate, because of the absence of mineralization, may cause oligotrophic lakes to become *dystrophic*. This is the reason for the presence of brown to dark-colored water in streams and lakes, called black water as mentioned earlier. Dystrophic water bodies are perhaps slightly less, if not equally, productive than mesotrophic ecosystems, but biologically more alive than oligotrophic lakes. The author notices from his surveys for soil and water sampling that the dystrophic swamps and lakes in southeastern United States are teeming with an assortment of wildlife. The dark-colored waters of the Okefenokee Swamp in South Georgia and the Everglades in South Florida are alive with aquatic wildlife from crawfish, blue gills, largemouth bass, and catfish to water moccasons and alligators at the top of the food chain. At one time, the American alligator was threatened with extinction, but at the end of the twentieth century its status as an endangered species has been lifted. The dystrophic water and favorable climate in South Florida are also reasons for the development of a lucrative aquaculture raising *Tilapia* for sale to restaurants and supermarkets throughout the continental United States. All these are provided to underscore the excellent biological productivity of dystrophic conditions.

The term dystrophic (Greek *dys* or *dvs* = bad, and *trophia* = nourishment) lake is believed by some to be a misnomer and they suggest naming it *humic lake* instead, which is allegedly more appropriate to describe the particular humus-rich condition (Hansen, 1962; Gjessing, 1994; Tulonen, 2004). In line with the suggestion above, the term *humic water* would perhaps also be more appropriate than the term *black water*, used by the present author for the dark-colored natural waters draining swamps and peat areas in the world. This would perhaps describe the colored conditions better, since more often than not the natural waters in the streams are only yellow to yellowish brown, hardly “black” enough to call it black. Moreover, the reason for such a discoloration is believed caused by the dominating presence of aquatic humic substances, and in particular fulvic acid, which in dilute solutions is usually yellow.

1.5.2 Effect on Hydrochemistry

Since this section is about chemical changes in water, the discussion is therefore more focused on AOM and its DOM fraction. However, as indicated earlier, the dissolved organic fraction is not only an important fraction of AOM, but DOM may also be present in aerobic soils. As indicated earlier, in normally moist condition, the soil may contain a sufficient amount of water for plant growth. Reinforcing the issue of this soil water, also known as soil moisture or soil solution (Tan, 2011), it should be realized that only some is present in the macropores as free water, whereas most of it is located in the soil micropores as capillary water, or as interstitial water bridging soil particles, or as intermicellar water in intermicellar spaces of clay minerals, and as hydration shells surrounding ions and soil clays in particular. All these locations where water is present may contain DOM, similar in quality perhaps as DOM in lakes and streams. However, not much is known about them and most, if not all, of the knowledge about DOM come from recent reports on AOM published by limnologists and biohydrologists.

It is obvious that AOM and in particular its DOM fraction is definitely affecting many chemical reactions occurring in the aquatic medium. The author believes that the term *hydrochemistry*, a new term introduced here replacing *water chemistry*, accommodates the trend of advancing science better and represents also accurately the chemical processes or chemistry in water than *geochemistry* or *biogeochemistry* preferred by geologists and even by a great number of soil scientists. The author agrees that these terms are indeed impressive, but they read or sound only more grandiose than the simpler down-to-earth name water chemistry. As discussed in the preceding section and stated above, AOM serves as the major source of food and energy for the aquatic microbial population, known in limnology as *decomposers*. Their activities and decomposition reactions are the start for the development of many biochemical processes and interactions in the liquid medium. By breaking down DOM components, elements are released and reused as nutrients by the decomposers as well as by the primary producers. The breakdown process was referred to earlier as *decomposition* in soil science, whereas the release and reuse of elements as nutrients are called *nutrient cycling*. However, the processes of decomposition in aquatic environments are different from those occurring in aerobic terrestrial ecosystems where adequate amounts of oxygen are present. Because oxygen is in low supply—dissolved O₂ in water amounts only 8 mg/L at 25°C—the breakdown of organic matter in natural waters proceeds along different pathways. The limited content of

free oxygen in water tends to develop *anaerobic conditions*, and the process of decomposition is known as *anaerobic decomposition*. It is often also called *fermentation*, where organic matter is not oxidized directly into CO₂ and H₂O as commonly occurring in aerobic decomposition. It often proceeds through formation of intermediate substances before the material is finally decomposed into CO₂ gas. For the principles and reactions of aerobic and anaerobic decomposition, reference is made to Tan (2011).

Another significant decomposition process recognized today is *photochemical degradation* of DOM, also called *photobleaching*, caused by solar radiation in the ultraviolet range. This is allegedly a spontaneous process capable of oxidizing DOM into substances grouped by limnologists under the name *dissolved inorganic carbon* or *DIC* (Bertilson and Tranvik, 2000; Miller et al., 2009). Since the degradation reactions of AOM yield new substances that may bring pronounced changes in the water chemistry, which in turn may influence other ecosystem processes, they will be examined in more detail in the following sections below. The author believes that photochemical decomposition may also occur with organic matter in soils, and especially with the DOM fraction in soil water. However, not much is published or known in the literature. Therefore, the examination below of the process is based on references from AOM, which perhaps may be extended to the DOM fraction in soils.

1.5.2.1 Photochemical Decomposition and Photobleaching

As indicated above, AOM can be broken down by the interaction with solar radiation (sunlight) in the ultraviolet (UV) range, which is known as *photochemical decomposition* or *degradation*. Although a number of scientists call it also photobleaching, this term seems to be used more in the sense of degradation of DOM, rather than the affected materials losing their color (Miller et al., 2009; Brinkman et al., 2003). However, the process is noted to decrease the optical density and absorptive capacity to light of the decomposed substances, which are properties related to color. Some of the hydrologists or limnologists call these types of DOM also *CDOM*, for *colored DOM* or *chromophoric DOM* (Klug, 2008; Conmy et al., 2004). The dark colors imparted onto the water medium diminish penetration of light, resulting in reduction in the growth of phytoplankton. Since in marine environments phytoplankton is responsible for photosynthesis, its reduction raises some concern about the possibility of harmfully affecting the production of atmospheric oxygen. The bleaching effect not only reduces absorption of light, but CDOM is reported to be broken down into low molecular weight

organic substances, possibly composed of damaged tissue that can be used for food by microorganisms.

Several different processes were reported responsible for the decomposition of aquatic organic material by photochemical reactions. These processes are, for convenience, divided by the present author into three major groups as follows: (1) direct processes, (2) indirect processes, and (3) transformation processes. The direct process involves oxidation by UV radiation of the sun, breaking down the AOM into carbon dioxide (CO_2) gas. This gas is soluble in water, hence is called DIC by Bertilson and Tranvik (2000). The solubility of CO_2 gas and its effects in water will be addressed in more detail in the section on inorganic and organic solutes below. Such a direct breakdown into CO_2 gas is considered by the authors above to enhance greatly the natural cycling of carbon. By the indirect processes, the bioavailability of AOM is increased, making it easier and more attractive for attack of the material by microorganisms. Especially the humic fraction in DOM is reported to be susceptible to this type of photochemical reactions, rendering it more labile to microbial degradation (Anesio et al., 2005). However, the explanations provided are somewhat confusing whether the amino acid and sugar components are released from the humic molecules or are just being exposed, making them more accessible to microbial attack. The authors also reported to have detected hydrogen peroxide (H_2O_2) as a decomposition product in addition to CO_2 . Hydrogen peroxide is known in chemistry as a powerful oxidation agent and in medical science as an effective disinfectant, hence its presence in natural waters will inhibit some of the microbial activities as also reported by the authors above. The third process, called transformation process by the present author but known by European limnologists as *photolysis*, involves the degradation of DOM and its transformation into formic acid, acetic acid, oxalic acid, malonic acid, and other low molecular weight organic acids. For some reasons, these acids are grouped as DICs by Bertilson and Tranvik (2000), although, by definition, DIC refers to inorganic types of carbon compounds, such as carbon dioxide (CO_2); carbon monoxide (CO); bicarbonates, e.g., $\text{Ca}(\text{HCO}_3)_2$ or the like; and carbonates, e.g., CaCO_3 or limestone; and several others. During the reactions, the processes discussed above also release a number of inorganic elements contained by the aquatic organic substances, thereby increasing the *nutrient element* pool. The authors above use the term *mineral nutrient* pool, but in soil science the term *mineral* refers to crystalline compounds, such as clay minerals, feldspar minerals, biotite minerals, quartz minerals, and the like. Releasing all the elements from the plant tissue requires a complete

breakdown without producing intermediate substances. Such a process is known in soil chemistry as *mineralization*, which is perhaps what is happening in the direct process. This process is therefore more likely the main nutrient element supplier of the pool. The other two processes terminate with the production of new substances or intermediate compounds, including the series of organic acids. What little amount in elements is associated at the time of decomposition will be released during the reaction, contributing in this way somewhat, but not much, to the nutrient element pool.

1.5.2.2 Anaerobic Decomposition and Respiration

Anaerobic decomposition is another important process by which AOM is broken down in aqueous media. Because the reactions occur under a limited supply of dissolved oxygen, hence are anaerobic in nature, reduction processes are more prevalent than oxidation reactions (Tan, 2011; Rich, 2003). Organic nitrogen is then generally reduced into NH_3 (ammonia) gas, and organic sulfur into H_2S (hydrogen sulfide) gas, whereas the carbon not used for food or cell production by the microorganisms is left to accumulate in the organic matter residue as detritus, humus, and/or humic substances. The energy required for the reactions above is normally generated by respiration and in this case by anaerobic respiration. The first step in this type of respiration process is the oxidation of organic matter according to the following simplified reaction (Tan, 2011):



Dissolved oxygen when still available, otherwise bound oxygen, such as in FeO , MnO , SO_4^{2-} , and NO_3^- , are the terminal acceptors for the electrons released. The pyruvic acid is broken down further into lactic acid as shown by the reaction below:



The reactions above indicate that the respiration processes of anaerobic organisms turn to fermentative pathways. Depending on the presence of specific microbial decomposers dominating the system, carbohydrates

or sugars can also be broken down into alcohol, methane, or other types of biogas. In the presence of yeast, the end results of anaerobic respiration are ethanol or alcohol and carbon dioxide, a process also called *ethanol fermentation*:



The four protons produced in reaction 1.1 may increase the hydrogen ion concentration of the humic or black water, as will also the dissociation of pyruvic and lactic acids. Therefore, the pH of the aqueous medium will decrease, which is the meaning of DOM acting as a proton donor as stated by many other authors (Leenheer and Croué, 2003).

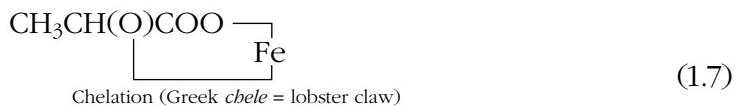
1.5.2.3 Organic Acids

The organic acids produced by decomposition of AOM are not limited to the two carboxylic acids in reactions 1.1 and 1.2 above. The decomposition reactions may perhaps yield a large variety of other acids, ranging from simple aliphatic acids to complex aromatic, heterocyclic acids, and humic and fulvic acids. In general, the humic substances form the bulk of all organic acids present and will be examined in more detail in later chapters of the book. This section intends to address only the nonhumic organic acids. Some examples of the simple organic acids have been mentioned in the preceding section above, e.g., formic acid, acetic acid, and several others. For a more complete list of possible nonhumic organic acids in terrestrial or soil's ecosystems, reference is made to Tan (1986, 2003a). This author also reported that even in soils, they were generally found in very small amounts. For example, a concentration in the range of 1.0 mmol/100 g soil is detected for acetic acid, and a content reported of 1×10^{-5} mol/L for the more complex acids, such as vanillic acids, pyruvic acids, and lactic acids in reactions 1.1 and 1.2. Many others of the organic acids are detected in even smaller concentrations, and because of these minute quantities they are unfortunately not considered of too much importance by many soil scientists. In contrast, although small in amounts, these organic acids have attracted considerable attention from limnologists, hydrologist, and biogeochemists alike due to their alleged effects on the biological health of the aquatic ecosystem.

The chemical reactivity of the nonhumified organic acids depends on the type and number of *active functional groups* in their structure. One group of these organic acids exhibits acidic characteristics attributed to their possession of only one or more $-\text{COOH}$ (carboxyl) functional groups. Formic acids, acetic acids, oxalic acids, and pyruvic acids in reaction 1.1 are some examples belonging to this group. Dissociation of the $-\text{COOH}$ groups releases the H^+ ions, affecting the hydrogen ion concentration of the solution. The remaining $-\text{COO}^-$ radical becomes negatively charged, enabling it to adsorb cations, providing to the organic compound a *cation adsorption*, hence a *cation exchange capacity*. Examples of the two chemical processes, respectively, are shown below



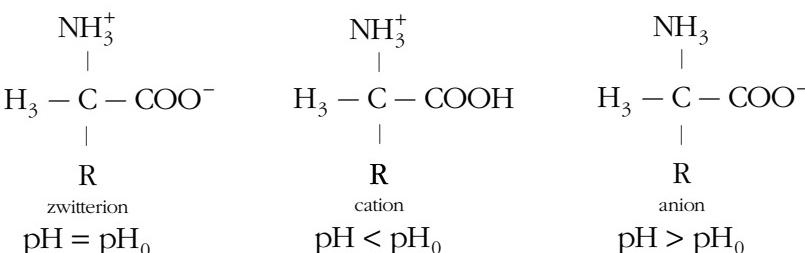
The second group of organic acids exhibits its chemical activity because of their content of $-\text{COOH}$ and also $-\text{OH}$ or phenolic-OH groups, e.g., lactic acid, citric acid and benzoic acid, and more. These substances are capable of dissociating the hydrogen ions from both the carboxyl and OH groups. Such a dissociation reaction creates two negative charges that can be used for interactions with cations by a process called *complex formation* or *chelation* as shown by the two reactions below



Reaction 1.6 is the author's classic example of a complexation reaction, known in soil science as *bridging* (Tan, 2011). It is as if the calcium ion forms a bridge between the two molecules of pyruvic acids. Currently, complexation is also defined as a *metal-ligand interaction*, where the metal—in this case calcium—is the electron-pair acceptor, and the ligands—pyruvic acids—are the electron-pair donors. The ligand is often also called the *sequestering agent* (Latin *ligare* = to bind). The second reaction, reaction 1.7, is an example of iron (Fe) chelated by lactic acid through the negative

charges created by the dissociation of the functional OH and COOH groups. The picture reflects the organic acid grabbing Fe with a pair of pincers or as if Fe is pinched by a crab claw. These bonds are stronger than those in binding cations through adsorption or cation exchange reactions. However, the ligands function only as the carriers moving the cations to new locations. When arriving at root surfaces, the chelated cations can often be exchanged with hydrogen (H^+) ions, although it is noticed that such an exchange occurs more rapidly with other cations within the series of the transition metals (Tan, 2011).

A third group of organic acids affecting the chemistry of the aqueous medium is the amino acid group. Free amino acids are also detected in small concentrations in humic water and as indicated earlier some people are therefore skeptical about their influence on the water chemistry. However, Wershaw et al. (2005) and Thomas (1997) have attached considerable significance to the presence of amino acids in natural waters. Thomas (1997) believes that free amino acids are not only the product of degradation processes, but can also be released by living aquatic organisms. The author above also indicates that their presence is generally positively correlated with base cations and biological activity in natural water bodies. This group of amino acids has been reported as important sources of food for the anaerobic microbial decomposers and producers (Tulonen, 2004; Wershaw et al., 2005). In most of the journal articles, they were mentioned without additional explanations about their effects on humic water. Amino acids are special because of their unique *amphoteric* character, which, depending on water pH, enables them to react with bases and acidic substances. This is one of the reasons for the positive correlations between free amino acids and cation concentrations in solutions noted by Thomas (1997). The chemical behavior of amino acids is usually explained by considering them *zwitterions*, which can be illustrated in a simplified form as follows below (Tan, 2003a, 2011):



The zwitterion structure is generally in the shape of a tetrahedron, in which R represents an H atom, a CH₃ group, or an aromatic carbon chain. At the isoelectric point, or more scientifically at pH = pH₀ (see Tan, 2003a, 2011), the amino acid species carries a positive and a negative charge, as shown in the diagram above, and is practically neutral. The term pH is then the pH of the surrounding water, whereas pH₀ refers to the pH at isoelectric point; in other words, it is the pH of water when the amino acid is practically neutral (0 charge). In “acidic” (pH < pH₀) medium, the large concentrations of H⁺ ions will neutralize the negative charge of the –COO⁻ group and the amino acid becomes positively charged, enabling it to react with anions, generally called *bases*. This is the driving force for what we call *anion exchange capacity (AEC)*. On the other hand, in “basic” medium (pH > pH₀), the large OH⁻ ion concentrations will neutralize the positive charges of the NH₃ group. The amino acid becomes negatively charged and can attract cations. This then provides the compound with a capacity to adsorb and exchange cations, called *CEC* or *cation exchange capacity*.

1.5.2.4 Organic and Inorganic Solutes

In chemistry and soil chemistry, *solutes* are, by definition, all the dissolved materials in solution. The soluble substances in a soil solution qualify then to be called solutes. The decomposition of AOM produces an assortment of substances, which are both soluble and insoluble in the aqueous medium. The soluble fraction is then the solute, which is chemically not different from the solutes in soil moisture or the soil solution. These solutes can be inorganic and organic in nature. They can also be divided further into noncharged and charged solutes. Many people also include inorganic and organic compounds in suspension, known as colloids, into the definition of solutes. As indicated earlier, Wershaw (2004) recognizes colloidal-DOM and “truly soluble” DOM. Among the soluble materials (or solutes), glucose and arabinose and other monomeric and polymeric sugars, glycerols, lipids, mannitol, and carboxylic acids have been cited above from reports of various national and international authors (Leenheer and Croué, 2003; Tulonen, 2004). Amino acids and organic acids are additional examples of soluble substances and were addressed in the preceding section above in some detail. The sugars dissolved in water are examples of noncharged organic solutes, whereas amino acids, when truly dissolved are charged organic solutes (zwitterions) and so are the other organic acids. The inorganic solutes comprising a great variety of inorganic acids, e.g., H₂SO₄, HNO₃, and H₃PO₄,

just to name a few as examples, are also electrically charged solutes when dissolved. Similarly as the organic acids, inorganic acids are also capable of dissociating their hydrogen ions into solution, thereby affecting the acidity of the water medium. The mineralization process of terrestrial and aquatic organic matter also releases a great number of ions, such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Fe^{2+} , Al^{3+} , Mn^{2+} , Cl^- , and more. As indicated before, many serve as nutrients for the terrestrial and aquatic organisms, and as true solutes, these ions behave differently than the colloids. Because of their ionic nature, they are smaller in size than the colloids, hence exhibit ionic behavior and do not have colloidal properties. Their chemical and electrochemical properties can be described using *chemical* and *electrochemical potentials* (Tan, 2003a, 2011). The charged solutes, and in particular the H^+ , Na^+ , K^+ , and other ions, serve as important counterions for the formation of *electric double layers* surrounding negatively charged complex inorganic and organic molecules and colloids such as clay minerals and humic matter. Such a process is not only of significance for maintaining electro-neutrality in soils and natural waters, but it is also affecting equilibrium and transport reactions in terrestrial and aquatic ecosystems.

In addition to the chemical compounds discussed above, natural waters also contain other soluble substances, which may or may not qualify to be called solutes. Free salts, CO_2 , O_2 , and other gases can also dissolve in natural waters (Tan, 2011). One of the gases often affecting natural waters is carbon dioxide (CO_2) from photochemical degradation of organic matter, microbial respiration, and from the atmosphere. The carbon dioxide is initially present in gas form, but depending on its partial pressure and other physical factors, this gas is soluble in water. Its concentration in solution is relatively low and amounts only 0.43 mg/L at 25°C (Tan, 2009). Nevertheless, it may have a significant effect on the acidity of natural waters. It may react with water to form carbonic acid or H_2CO_3 . As a weak acid, H_2CO_3 can dissociate its hydrogen ions, affecting in this way the pH of the aquatic medium:



Another important gas is atmospheric oxygen, which is also soluble in water (Tan, 2009). This is perhaps one example of a controversial topic

where the issue depends on the willingness of the reader to accept dissolved oxygen as a solute or not. By definition, it is in solution and its content is higher than that of dissolved CO₂ gas. However, at a maximum concentration of 8 mg/L (at 25°C), the dissolved amount is considered just sufficient for sustaining the aquatic life in natural waters. This dissolved oxygen is vital for the survival and growth of many aquatic organisms, especially fish. Unfortunately, the low content of dissolved O₂ tends to be consumed rapidly by the so-called biological oxygen demand (BOD), and AOM falls within this BOD concept since it can be considered as a contaminant. Eutrophication, resulting in excessive growth of aquatic weeds, is another process taking a severe toll on the limited supply of dissolved oxygen, usually to the point of killing all the fish and crustaceans.

1.6 Issue of Acronyms

As noticed from the preceding sections, a great number of acronyms have been applied for rapid and ease of identification of the various types of organic materials. A list of the most commonly used acronyms is provided in Table 1.1. The proliferation of creating freely any acronyms as scientists see fit is often amusing, if not confusing, and may be subject to lots of arguments. Especially, geochemists and hydrologists, studying AOM and DOM, have made use of acronyms, disregarding that many of the names created can also apply for other different types of materials. The following examples illustrate the disturbing nature of some of the issues. For example, the use of SOMM may truly confuse many soil scientists the way it is defined and reported by the creators. The acronym apparently only means *soil organic matter model*, and referring it as “the SOMM model” not only raises the question as to the necessity of creating such an acronym, but also on the appropriateness of stating “soil organic model model.” Another controversial example is the use of SOM for *soil organic matter*, which can easily refer also to *soluble organic matter*. Since in the author’s opinion soluble organic matter is in fact dissolved organic matter, SOM can then mean DOM. Because dissolved organic matter is present naturally in river and lake waters, some of the hydrologists often use the acronym NOM, instead of DOM, for its identification (Wershaw et al., 2005). However, SOM, TOM, and POM, being formed and present naturally, also qualify to be called NOM. The acronym TOM, intended to be used for *total organic matter*, can also refer to *terrestrial organic matter*, and so on. Several other examples

Table 1.1 Acronyms Cited by References for Identification of Terrestrial and/or Aquatic Organic Matter

| Acronym | Full Name | Reference |
|---------|--|-------------------------------|
| BDOC | Biodegradable dissolved organic carbon | Tulonen, 2004 |
| CDOM | Chromophoric dissolved organic matter | Quaranta, 2011 |
| CDOM | Colored dissolved organic matter | Klug, 2008; Conmy et al. 2004 |
| DIC | Dissolved inorganic carbon | Bertilson and Tranvik, 2000 |
| DOC | Dissolved organic carbon | Tulonen, 2004 |
| DOM | Dissolved organic matter | Leenheer et al., 1998 |
| EOC | Extracellular organic carbon | Tulonen, 2004 |
| LDOM | Labile dissolved organic carbon | Tulonen, 2004 |
| NOM | Natural organic matter | Wershaw, 2004 |
| OM | Organic matter | Dubnick et al., 2010 |
| POC | Particulate organic carbon | Tulonen, 2004 |
| POM | Particulate organic matter | Dubnick et al., 2010 |
| RDOC | Refractory dissolved organic carbon | Tulonen, 2004 |
| RDOM | Refractory dissolved organic matter | Tulonen, 2004 |
| SOM | Soil organic matter | |
| SOMM | Soil organic matter model | Chertov and Komarov, 1997 |
| TOC | Total organic carbon | |
| TOM | Total organic matter | |
| WEOM | Water-extractable organic matter | Bolan et al., 2011 |
| WSOM | Water-soluble organic matter | Bolan et al., 2011 |

can be provided, but as can be noticed, the examples above are perhaps sufficient to illustrate how confusing the issue is and how it can make one's head spin. Apparently, standardization for the creation and use of acronyms is needed. Such a standard approach may perhaps avoid authors from using several different acronyms for a similar substance and bring some order in the creation and use of acronyms.

Chapter 2

Concept of Humus

2.1 Issue of Humus

As discussed in the preceding chapter, soil organic matter is a term frequently used to indicate the dead organic fraction only, and the live fraction, although of equal importance, is usually ignored. This dead organic fraction was divided in the previous chapter into two groups: a group of organic matter at various degrees of decomposition, related to litter, and another group composed of completely decomposed materials, which was identified as humus. The first group mentioned above, containing most of the undecomposed material, has been discussed in the preceding chapter. The present chapter is intended to continue by now examining the concepts and issues of the second group of organic matter, known as humus. The term *humus* (Latin for *soil, earth, ground, or vegetation*) is a name that seems to be accepted by many scientists from the early days until late in the twentieth century for organic residues, composed of brown to black amorphous materials with no traces of any cellular structures of the original plant and animal materials. *Merriam-Webster's Dictionary* also defines humus as a "brown or black to complex variable material resulting from partial decomposition of plant and animal matter and forming the organic portion of soils" (Merriam-Webster, 1973). The nature of this highly complex system, called humus, is still confusing until today. Most people in the past seem to associate it with the NaOH-extractable fraction of soil organic matter (Russell and Russell, 1950; Whitehead and Tinsley, 1963). However, this alkali-soluble part is, by definition, the soil humic substances, and the example of Waksman's ligno-protein compound presented as evidence of humus by Whitehead

and Tinsley (1963) is only adding to the confusion because this is the classic polymer concept of humic acid. Considering humus as equivalent to humic matter was very common in those years, and even Flaig (1975) and more recently Haider (1994), prominent authorities on the subject, use the terms *humus* and *humic matter* interchangeably. Kumada (1987) adds to the confusion by using in his book the terms SOM (soil organic matter) and humus synonymously, whereas Schnitzer's (2000) statement that he, personally as a SOM scientist, prefers the use of the term SOM for humic substances, makes the issue more confusing. Apparently, the old ideas seem to convey the message that humus is, in essence, nothing more than humic matter, which seems to boil over into the modern ideas of the new millennium. Such a concept is subject to many arguments since it ignores the materials extracted along and that are discarded during purification of the humic substances. Then, there are substances that are not extracted and remain in the residue, whose origin came from the sample defined as humus. The definition agreed on by most scientists also spells out that humus refers to all organic plant materials that have been decomposed to an "unrecognizable" amorphous mass. Page (1930) suggests dropping the term humus and replacing it with the names *nonhumic* and *humic matter*. Page groups fulvic acid and colorless decomposition products of organic matter under the name nonhumic matter. The dark or black materials are grouped as the humic fraction. However, Waksman (1938) proposes just the opposite in his famous book—to drop the names of nonhumified and humified fractions altogether and just use the name humus for soil organic matter. Failure to find a satisfactory agreement in the following years has resulted in a lot of confusion on the nature of humus. The present author suggests retaining the name humus and dividing it into nonhumified and humified fractions (Tan, 1986). Whether this idea caught on is perhaps conjecture, because since then similar definitions have surfaced in the more modern literature recognizing humus to be composed of a nonhumic and a humic fraction (Brady and Weil, 2008; Stevenson, 1994). Wershaw (2004) calls this "mixture"—known as humus—NOM (natural organic matter), apparently following Stevenson's (1994) idea, which considers soil organic matter identical to humus. In his book, Stevenson lists the definition of soil organic matter as "same as humus" (Stevenson, 1994; *Humus Chemistry*, see table 2.3, p. 33). However, Stevenson's idea cannot be considered original or new because it is an adaptation, if not a quotation, of an older concept developed by Waksman (1938). As indicated earlier, it was in fact Waksman who originally proposed using the name humus only for referring to soil organic matter.

It is against this backdrop of controversies that in continuation of the previous chapter on organic matter, this chapter is written to examine humus as the basic link of organic matter to humic substances. The concepts and issues of humus, surrounding its division into nonhumic and humic fractions, will be covered below, starting first with an examination of the nonhumic fraction in some more detail, followed later by the humic fraction.

2.2 Nonhumic Fraction of Humus

From the above discussion, it is perhaps clear that by current standards, humus is distinguished into a nonhumified and a humified fraction (Tan, 2011; Stevenson, 1994). The nonhumified fraction is treated here as a revised and extended version of Page's (1930) definition of nonhumic matter. However, it excludes fulvic acids, which are now grouped with the humified fraction. The nonhumic matter is now defined to include all substances released by decomposition of residues of plants and other organisms. This part of humus is believed to contain, in general, most, if not all, of the biochemical compounds synthesized by plants and other soil organisms. Most of them are in the forms of identifiable chemical substances, with definite physical and chemical characteristics, e.g., carbohydrates, amino acids, protein, lipids, waxes, nucleic acids, lignin, and many other organic compounds that make this nonhumic fraction different from the humic fraction. After their release, these substances in question are usually subject to further degradation and decomposition reactions, and are the main sources for the synthesis or formation of the humic fraction by a process called *humification*. Often, they are adsorbed by the inorganic soil components, such as clay, and they may also occur under anaerobic conditions. Under these conditions, the compounds above will be temporarily protected from further decomposition reactions, enabling their accumulation in soils.

This part of soil humus is often ignored, but is in the author's opinion equally important as the other part of humus called the humic fraction. It is an essential part of humus because it is not only the source for the formation of humic substances, as indicated above, but also the primary source of food and energy for the soil population of microorganisms. The nonhumic fraction is produced first, and without it there will be no humic matter formed. Decomposition of the plant remains usually starts with the decay of sugars and starches, the carbohydrates produced originally by

photosynthesis. Cellulose, proteins, and lipids then follow as invasion of the dead tissue by detritivores increases. Lignin is believed to be the most resistant, but can also be broken down quickly in the presence of white-rot fungi. The reaction processes can be purely chemical or abiotic, such as hydrolysis, or they can occur through microbial actions. As the degradation of plant remains continues, the substances in question may be broken down further or modified by soil microorganisms. The more complex substances, e.g., lignin, lipids, and proteins, are transformed into simpler or smaller compounds, providing easier access to soil microbes metabolizing them. All of the substances above are then released into the soil, forming what is called above the nonhumic fraction of soil humus. It is a very complex mixture of components, some only slightly different from the substances originally present in the plant tissue, whereas others have been changed or produced by the soil microorganisms, as discussed earlier. The estimate is that around 30% of humus is composed of the nonhumic components (Stevenson, 1994; Brady and Weil, 2008). Many believe that lignin makes up the bulk of the nonhumic fraction, whereas carbohydrates are second in amount, followed by substantially lower quantities of the other substances (Tan, 2011). However, using ether as the extraction reagent, Waksman (1938) noted that lignin humus made up 35–50%, soil protein 30–35%, hemicellulose 5–12%, and cellulose 3–5% of the humus fraction in general. Although many of the nonhumified humus components are present only in minute amounts, often difficult to analyze, all of them are important for the health of the ecosystem. When by the end of the twentieth century, humic research turns to also examining dissolved organic matter, called DOM by hydrologists, and aquatic organic matter, as discussed in Chapter 1, the presence of carbohydrates in aquatic and terrestrial ecosystems seems to become more prevalent than that of lignin. Indigenous aquatic plants, especially, do not need lignin for growth and development, and release after cell death their photosynthates, mainly monomeric and polymeric sugars, in the surrounding medium. Leakage across cell membranes and active secretion are additional processes enriching the aqueous medium with a variety of carbohydrates and other metabolically produced substances. The ubiquitous presence of carbohydrates in aquatic and terrestrial systems is obvious. In view of the above, the difference can perhaps be stated that aquatic humus is characterized by the presence of carbohydrates, whereas terrestrial humus is dominated more by high lignin contents, as also claimed by Waksman (1938). The issue has also been addressed in Chapter 1 where aquatic organic matter and terrestrial organic matter were recognized as two separate groups.

To avoid noisy arguments or criticisms, it is perhaps necessary to add that the author also realizes the need for many of the chemical substances to react during the decomposition processes, generating abiotically and/or biotically the so-called humic substances; however, this is another story that will be discussed in Section 2.3. The current section is about the nonhumic fraction only, and will continue below by examining several of the major components, starting with carbohydrates because they are widely distributed in terrestrial and aquatic ecosystems as noted above. This is then followed by lignin, considered by most scientists the most abundant humus component in terrestrial systems, and several of the others will be addressed next. Their functions and importance in relation to the formation of humic substances will be examined briefly. Some basic elements of biochemistry will be included for reasons explained below. Many are often confused about what biopolymers are, what “aromatics” mean, and what the difference is between phenol and quinone. Even some hardcore scientists wonder about terms such as phenolic-OH and the like, which is evident from criticisms in the form of alleged improper use of terms, often fielded to the author. Therefore, some of the basic structural concepts need to be included to explain the chemical behavior of the compounds, which is necessary in understanding the reactions and interactions in humic matter formation. The materials provided can serve as background information, which it is hoped will increase the reader’s comprehension of the issues of humic substances discussed later.

2.2.1 Carbohydrates

Carbohydrates are perhaps the most important constituents of plants. They are considered one of the three major groups of food substances, with the other two being protein and oil. Carbohydrates are compounds yielding polyhydroxy aldehydes or ketones upon hydrolysis. Polyhydroxy aldehyde or aldose is the scientific name of glucose, which we call sugar, whereas ketose is the scientific name of fructose, another type of sugar (Figure 2.1). Carbohydrates are synthesized first by green plants through a process called photosynthesis, after which production of lignin, protein, and oil then begins. In living plants, carbohydrates serve as sources of energy for many biological functions, and play an important role in the synthesis of nucleic acids, lignin, and other structural components in plant tissue, in addition to protein and oil.

The carbohydrate compounds are more controversial than protein and amino acids in the issue of humic matter formation. For a long time, they

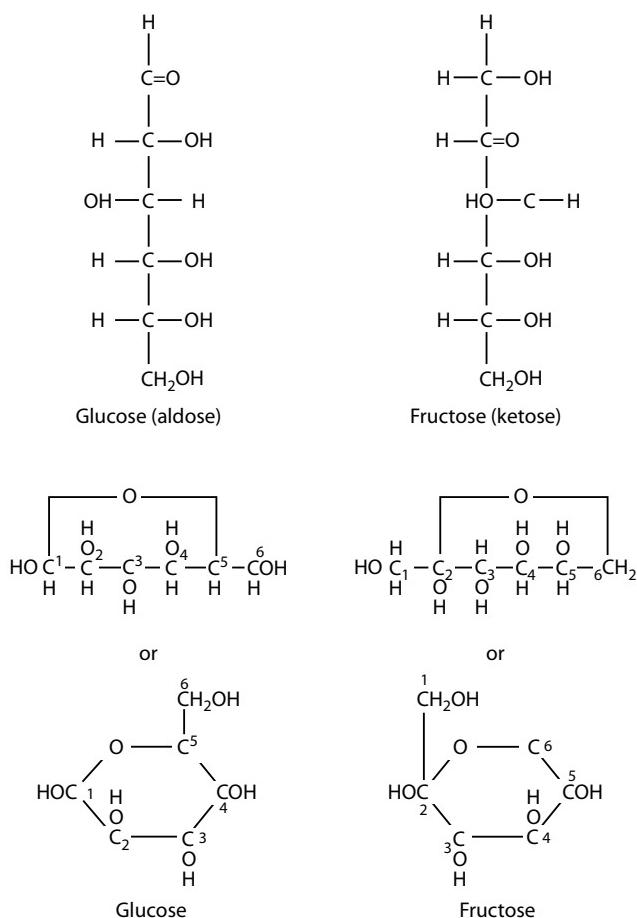


Figure 2.1 Glucose and fructose open-chain (top), ring and cyclic structures (bottom), showing differences in being an aldose and ketose, respectively, due to the position of the carbonyl (C=O) group in the carbon chain.

were regarded as contaminants rather than as precursors of humic matter. At the beginning of the twentieth century, Maillard's (1916) revelation that humic matter can be synthesized from simple sugars, e.g., sucrose, compelled many scientists to start reviewing the idea of carbohydrates as possible building constituents of the humic molecule. Maillard's abiotic theory of the synthesis of humic matter from sugar is known today as the *Maillard reaction*. However, it was the discovery of aquatic humic matter that has propelled the role of carbohydrates as major contributors in the formation of humic matter. The concept of aquatic humic matter, and in particular of marine and autochthonous aquatic humic matter, is based on a carbohydrate–protein combination (Nissenbaum and Kaplan, 1972; Hatcher

et al., 1985). The hypothesis was presented that this aquatic humic matter is a sugar–amino acid condensation product, although some regard it as being derived by autoxidative cross-linking of unsaturated lipids from plankton (Harvey and Boran, 1985). In terrestrial humic matter, polysaccharides have been identified earlier as important components of fulvic acids, whereas hymatomelanic acid is believed to contain polysaccharides bonded by ester linkages (Tan and Clark, 1968; Clark and Tan, 1969; Tan, 1975). However, today, many favor the theory of supramolecular self-assembling or spontaneous aggregation of carbohydrate molecules in the formation of humic substances, which will be addressed later in the book.

2.2.1.1 Saccharides

The carbohydrates, also called saccharides, are scientifically distinguished into three groups of saccharides: (1) monosaccharides, (2) oligosaccharides (Greek *oligos* = few), and (3) polysaccharides. The monosaccharides are the simple sugars, such as glucose and fructose, whereas the oligosaccharides are compound sugars composed of 2 to 10 monosaccharides. Like our table sugar, a disaccharide, they are soluble in water and sweet to taste (Figure 2.2). On the other hand, polysaccharides are complex carbohydrates and are composed of many (10 or more) types of sugars or monosaccharides. They are sometimes distinguished into homopolysaccharides and heteropolysaccharides. The homopolysaccharides are composed of repeating units of the same monosaccharides, whereas the heteropolysaccharides are made up of different monosaccharides. Some of the units, bonded together by glucosidic bonds, are glucose, xylose, and arabinose. Starches, cellulose, and

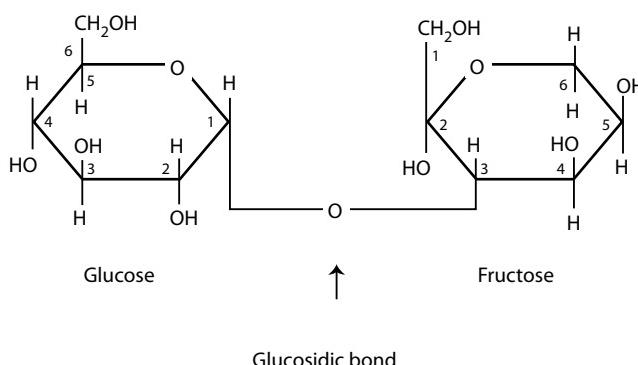
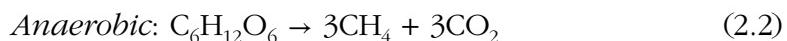
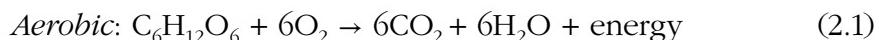


Figure 2.2 Example of a disaccharide, composed of glucose and fructose, held together by a glucosidic bond.

hemicellulose are examples of polysaccharides, and as such are not called sugars. They are usually amorphous and tasteless, and disperse in water to form colloidal suspensions. For more details on the basics and chemistry of saccharides or carbohydrates, reference is made to Tan (2011).

2.2.1.1.1 Monosaccharides and Oligosaccharides

Since carbohydrates are also the principal foodstuffs for soil microorganisms, they are rapidly attacked by the soil microbial population. The simple sugars and the disaccharides are the preferred source of materials, and are subject to anaerobic and aerobic decomposition reactions. In the aerobic process, the sugars are broken down completely into CO_2 and H_2O , while the energy released is used by the microbes for growth and performing other biological functions. In the anaerobic process, the sugar is broken down into CH_4 , methane, and CO_2 . The decomposition processes can be illustrated by the reactions below:



A partial decomposition is also possible by microbial fermentation, resulting in the production of ethyl alcohol ($\text{C}_2\text{H}_5\text{OH}$). This process can be illustrated as follows:



The relatively rapid decomposition as discussed above may indicate that most of the simple sugars have to be broken down before they can be used for the formation of humic matter. Although it appears that microorganisms have the advantage in the competition for sugars between microorganisms and humic acid synthesis, a substantial amount of the sugars may in fact escape decomposition. Some may be adsorbed in intermicellar spaces of expanding clay minerals, rendering them inaccessible to enzymatic attack, whereas others may enter into complex combination with toxic metals, making them less susceptible to microbial attack. Additional monosaccharides and oligosaccharides can also be produced by the decomposition of polysaccharides that are next in line in the degradation process. The resistance of polysaccharides to enzymatic attack by microorganisms depends on a number of factors. Polysaccharides are known to be able to form branch-like

structures, and the greater the amount of branching, the greater will be the resistance to enzymatic degradation.

2.2.1.1.2 Soil Polysaccharides

Soil polysaccharides may be different from the original plant polysaccharides discussed above. Some of them can be produced by soil microorganisms, whereas others are believed to be formed *in situ* (in the soil) from the partial degradation products of plant polysaccharides and free monosaccharides. The latter are derived from the decomposition of plant and microbial residues. Polymerization of these degradation products and of the free monosaccharides is reported to yield polysaccharides that are very heterogeneous and highly branched in structure. Linkage is believed to be induced by enzymes released during autolysis of microbial cells, and the “new” polysaccharides are considered even less susceptible to biodecomposition than their plant counterparts (Stevenson, 1994; Martin et al., 1975). However, some people feel that the resistance of soil polysaccharides to microbial attack is due more to adsorption by clay minerals and chelation with toxic metals than to complex molecular structures (Cheshire et al., 1977). Regardless of the differences in opinion, this resistance is one of the reasons why polysaccharides can accumulate in soils, although their concentrations rarely amount to more than 20% in soil humus. These soil polysaccharides then serve as additional building materials for the synthesis of humic compounds. However, the opinion is present that all these carbohydrates are not considered parts of the humic molecule core. Several scientists believe that they are important only as attachments to peripheral side chains of the humic molecule. Such an idea may perhaps be true for terrestrial conditions; however, with the advanced knowledge of aquatic organic matter (see Chapter 1), the nature of aquatic humic substances is more likely to be carbohydrate–protein systems rather than ligno–protein complexes or aggregates.

Amino sugars are currently added to the carbohydrates as possible precursors of humic acids. Biologically resistant complexes are formed by reaction with lignin and phenols. The nature of amino sugars related to formation and other issues of humic substances is addressed briefly in the next section below.

2.2.1.2 Amino Sugars

These compounds are simple sugars with substituted amino groups in their carbon chains. The most common form of an amino sugar is glucosamine,

found as a component of mucopolysaccharides and glycoprotein present in saliva and eggs (Conn and Stumpf, 1967). Glucosamine-like substances have also been detected in the mucous layer encasing bacterial cells. Galactosamine has also been mentioned as an important amino sugar in soils. It is an epimer of glucosamine, differing from the latter only in placement of an OH group in the carbon chain.

According to Stevenson (1994), amino sugars have often been mistakenly referred to as *chitin*, the material of the hard shell of insects and crustaceans. Although chitin exhibits a basic molecular structure almost similar to glucosamine, it is in fact a polymer of *N*-acetyl-D-glucosamine. Perhaps the name chitin is confused with the term *chitosan*, which is indeed a polymer of glucosamine (Martin et al., 1975). This then may provide some justification as to why chitosan can be used as a general name for amino sugars. To explain more clearly the differences and similarities between glucose, glucosamine, chitosan, and chitin, the molecular structures are presented as illustrations in Figure 2.3. Since the structures of simple sugars can be written in several ways, three types of structures for glucose are given in Figure 2.1: (1) open-chain, (2) ring, and (3) cyclic structures. In aqueous solutions, it is noted that equilibrium exists between the forms with an open-chain and a ring or cyclic structure (Gortner, 1949; Tan, 1998).

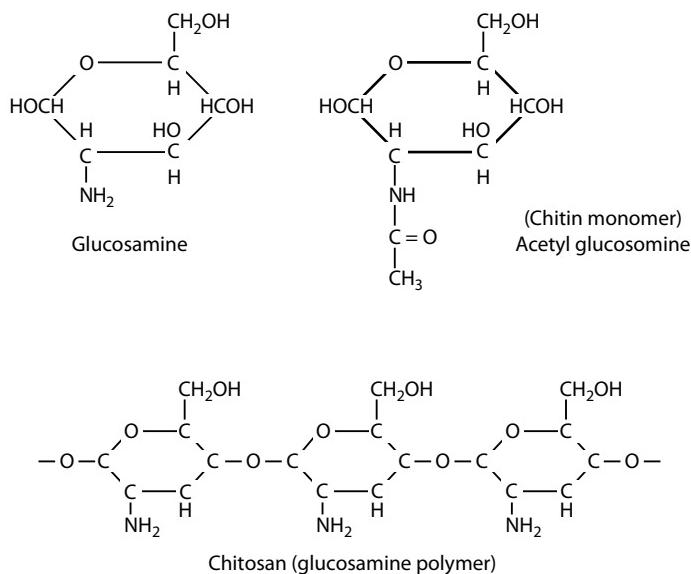


Figure 2.3 Molecular structures of glucosamine, chitin, and chitosan, showing their relations with glucose (some of the H and OH are not drawn due to space limitations).

The amino sugars are believed to serve several functions in soils. They are important sources of nitrogen for plant and microbial life, and affect the physical and chemical conditions of soils. From the standpoint of soil physics, mention has been made in the literature of interaction reactions between amino sugars or polysaccharides and soil mineral particles encouraging soil aggregation, hence formation of stable soil structures beneficial for plant growth and the environment (Greenland et al., 1961, 1962; Baver, 1963). Currently, amino sugars are also considered important components for the synthesis of humic matter. They can enter into reactions with phenols and quinones to form a basic humic molecule. In the abiotic Maillard reaction, glucosylamine is produced first, leading to the formation of *melanin*, a dark brown to black aromatic plant pigment found widespread in the natural environment (Ziechmann, 1994). The disintegration products are called *melanoids*. Some biochemists consider melanin to be a chromoprotein, the colored protein of certain seaweed and the material in black wool and hair of animals (Gortner, 1949). Whatever the nature is, melanin and melanoid are assumed to be important precursors in the synthesis of humic acids by a process sometimes also called the melanoidin pathway (Nissenbaum and Kaplan, 1972; Hatcher et al., 1985).

2.2.2 Lignin

Lignin is a system of thermoplastic, highly aromatic polymers of the phenylpropane group. The name is derived from the Latin term *lignum* (= wood). It is one of the three major components of wood, with the other two being cellulose and hemicellulose (Schubert, 1965). The bulk of lignin occurs in the secondary cell walls where it is associated with cellulose and hemicellulose. It is noted to coexist with the cellulosic plant components in such an intimate association that its isolation requires drastic chemical treatments that often alter the structure of lignin itself. The latter raises questions about the assumption held by most biochemists that lignin is associated physically, rather than chemically, with the polysaccharides. The nature of the lignin–polysaccharide complex still has to be resolved, and more definite data need to be presented refuting one or the other, or supporting the presence of both physical and chemical interactions.

The quantity of lignin increases with plant age and stem content. It is not only an important constituent of the woody tissue, but it also contains the major portion of the methoxyl content of the wood. A large amount of lignin is also detected in the vascular bundles of plant tissue. The purpose is perhaps to strengthen and make the xylem vessels more water resistant. By

virtue of the presence of larger amounts of vascular bundles, the lignin content of tropical grasses is considerably larger than that of temperate region grasses (Tan, 2011; Minson and Wilson, 1980). Consequently, soils under tropical grasses are expected to have higher lignin contents than soils under temperate region grasses. These differences may produce differences in the nature of humic substances formed.

2.2.2.1 Lignin Monomers

The building stones of lignin are monomeric lignin possessing a basic phenylpropane carbon structure. Three types of lignin monomers can be distinguished on the basis of the type of wood or plant species, e.g., coniferyl, sinapyl, and β -coumaryl monomers (Figure 2.4). The coniferyl type characterizes lignin in softwood or coniferous plants and the sinapyl type represents lignin in hardwood, whereas the coumaryl type is typical of lignin in grasses and bamboos. Several of these monomers are linked together to form the total lignin polymer. The process, called *polymerization*, forms a very complex and long series of a lignin polymer structure (see Tan, 2000).

2.2.2.2 Aromatization

The ultimate source for formation of lignin is carbohydrate or intermediate products of photosynthesis related to carbohydrates. The process of conversion of the nonaromatic carbohydrates into substances containing phenolic

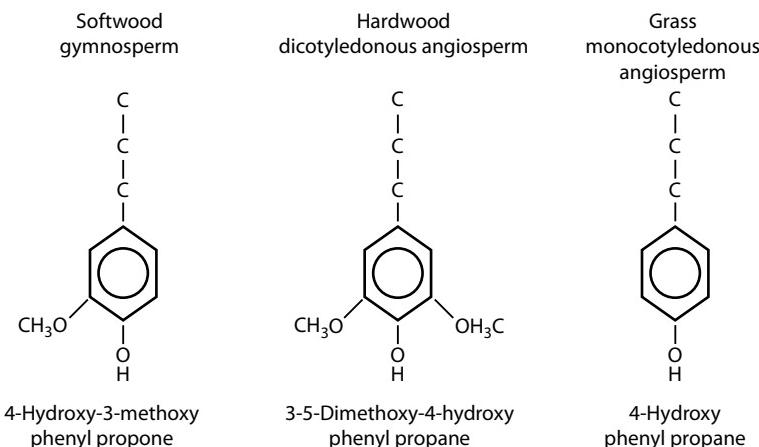


Figure 2.4 Lignin monomers from softwood, hardwood, and grass or bamboo.

groups characteristic of lignin is called *aromatization*. Enzymatic reactions are required to effect such a drastic transformation of nonaromatic carbohydrates into aromatic precursors of lignin. Several theories have been advanced on the aromatization process, e.g., aromatization of carbohydrates through a *dehydration process* and the *shikimic acid pathway*.

In the dehydration theory, carbohydrates, such as fructose, release three water molecules, and with the assistance of enzymatic reactions, three possible aromatic end products are produced, e.g., pyrogallol, hydroxyhydroquinone, phloroglucinol, or a combination thereof (Figure 2.5).

The shikimic acid pathway (Figure 2.6) has been adopted from the theory for the biosynthesis of aromatic amino acids from carbohydrate precursors with the help of enzymes originating from *Escherichia coli* bacteria (Schubert, 1965). The end products, phenylpyruvic acid and *p*-hydroxyphenylpyruvic acid, yield, by transamination reactions, phenylalanine and tyrosine, respectively. As illustrated in Figure 2.6, the chemical structures of these compounds show close similarities to those of the monomeric units of lignin. In particular, the structure of *p*-hydroxypyruvic acid is almost the same as that of

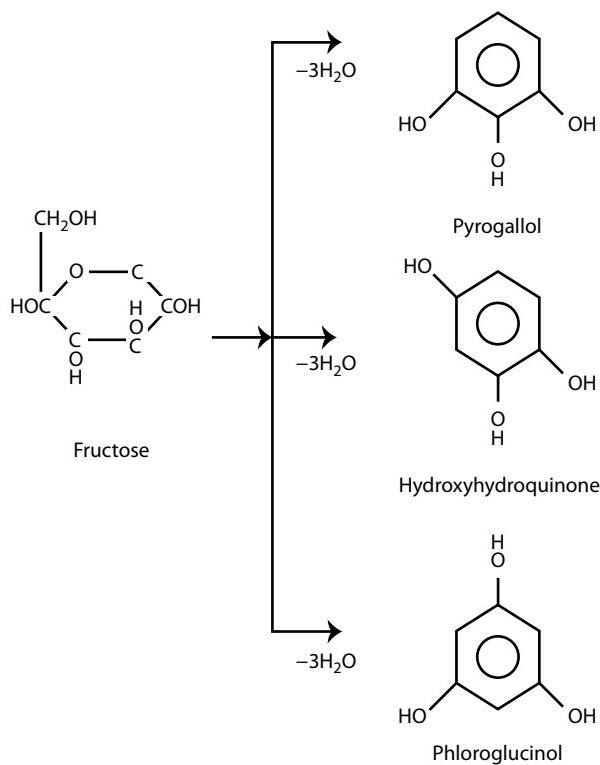


Figure 2.5 Aromatization of fructose through a dehydration process.

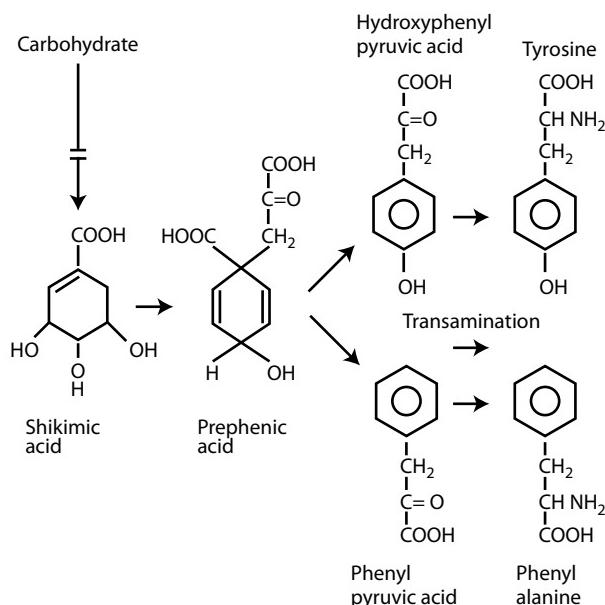


Figure 2.6 Bioformation of compounds in the shikimic acid pathway with molecular structures similar to lignin monomers.

β -coumaryl lignin, leading to the assumption that lignin monomers may have been formed through similar processes. In addition, the structures of phenylalanine and tyrosine are also similar to those of ligno–protein compounds, the humic substances according to the ligno–protein theory. These findings have an important bearing on the processes in the synthesis of humic substances, which will be discussed in more detail in one of the following sections. The similarities apparently support the hypothesis that plant biopolymers can be transformed into humic substances without drastic structural changes.

2.2.2.3 Lignification

In the growth of woody plants, carbohydrates are synthesized first. The formation of lignin then begins, and the spaces between the cellulose fibers are gradually filled with lignified carbohydrates. This process is called *lignification* and serves several functions:

1. It cements and anchors the fibers together.
2. It increases the resistance of the fibers to physical and chemical breakdown.
3. It increases the rigidity and strength of cell walls.

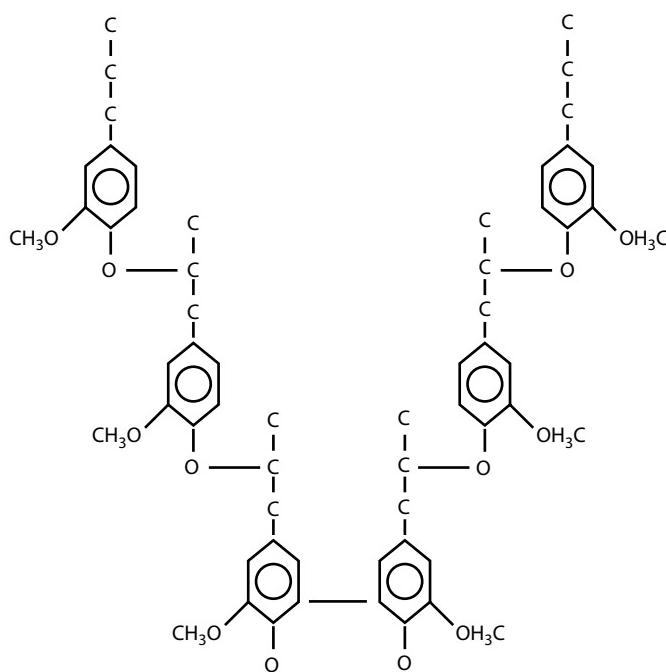


Figure 2.7 Hypothesis for a softwood lignin structure by a systematic linkage of coniferyl alcohol monomers.

In the process, the lignin monomers are bonded together by a process called polymerization, to form a complex chain of large lignin molecules (Figure 2.7). It is believed that after lignification, the lignified tissue then no longer plays an active role in the life of plants, but serves only as a supporting structure. Nonlignified plant parts contain more moisture, are soft, and break more easily.

2.2.2.4 Decomposition of Lignin

Lignin is insoluble in water, in most organic solvents, and in strong sulfuric acid. It has a characteristic UV absorption spectrum and gives characteristic color reactions upon staining with phenols and aromatic amines. It hydrolyzes into simple products, as do the complex carbohydrates and proteins. When oxidized with alkaline benzene, it produces up to 25% vanillin.

Lignin is considered an important source for the formation of humus, and especially humic matter. The high resistance of lignin to microbial decomposition is perhaps the reason why it accumulates in soils. It is believed that, depending on the condition, this could result in the formation of peat,

which in time can be converted into kerogen, coal, and ultimately oil (fossil fuel) deposits. Nevertheless, lignin can be attacked by very specific micro-organisms in the group of Basidiomycetes (Schubert, 1965; Paul and Clark, 1989). Several forms of these so-called lignolytic fungi have been reported as the major organisms responsible for the partial decomposition of lignin, e.g., white-rot, brown-rot, and soft-rot fungi. In well-aerated soils, the white-rot fungi are reported to decompose wood containing lignin into CO₂ and H₂O. Patches of a white substance are often formed in the residue, hence the name white rot. These white patches have been identified as pure forms of cellulose. According to Paul and Clark (1989), the brown-rot fungi are useful for the removal of the methoxyl, –OCH₃, group from lignin, leaving the hydroxyphenols behind, which upon oxidation in air produce a brown color. However, Schubert (1965) believes that the cellulose and other associated carbohydrates are attacked preferentially, leaving the lignin behind, which turns the residue brown. The soft-rot fungi are most active in wet soils and are specifically adapted to decomposing hardwood lignin.

The hydroxyphenol units resulting from demethylation of lignin by white-rot fungi can be oxidized to form *quinones*. The latter are believed to be capable of reacting with amino acids to form humic substances (Flaig et al., 1975). Lignin itself has the capacity to react with NH₃. The process, called *ammonia fixation*, has been applied in industry for the production of nitrogen fertilizers by treatment of lignin and other materials rich in lignin, e.g., sawdust, and peat, with NH₃ gas. The exact mechanism of fixation is still not known, but it is believed that NH₃ reacts with the phenolic functional groups in lignin.

2.2.3 Phenols and Polyphenols

Phenols are aromatic carbon compounds with a general formula of C₆H₅OH. They are derived from benzene, C₆H₆, by replacing one or more of the hydrogens with OH. Benzene, a flammable colorless compound, is called aromatic because of its characteristic structure of six carbon atoms linked by alternate single and double bonds in a symmetrical hexagonal configuration. The C₆H₅ group in phenol is called the *phenyl group*, from the Latin term *phene* (= shining), because burning benzene produces a very bright light.

By linking several monomeric phenols together, polyphenols are produced. As indicated earlier, the phenols and polyphenols can be derived from two sources: from the decomposition of lignin and from the synthesis by microorganisms. Stevenson (1994) believes that uncombined phenols are present in higher plants in the form of glucosides and tannins.

2.2.3.1 Lignin-Derived Phenols and Polyphenols

Biodegradation of lignin has been implicated in producing phenols and polyphenols. Specific types of fungi have been discovered capable of attacking lignin, a compound that is generally resistant to microbial decomposition. In addition to the Basidiomycetes referred to earlier, another group, the Ascomycetes, has also been mentioned as important lignin-degrading organisms (Schubert, 1965). These organisms attack lignin by excreting enzymes in the phenoloxidase group, which can be distinguished into two basic types of enzymes: tyrosinase and laccase.

The mechanism of phenol formation from lignin is, in essence, the reverse process of lignin synthesis. Complex diagrams have been presented by a number of authors showing pages of flow sheets illustrating the degradation of lignin into its monomeric type, which through a labyrinth of successive reactions is broken down into phenols (Haider et al., 1975; Schubert, 1965). A shorter and less complex diagram has been presented by Flraig (1966, 1975). To avoid confusion by presenting these complex diagrams as is done in many other books, and to underscore the purpose for better comprehension by a variety of readers, a simple diagram is provided in Figure 2.8 as the present author's version of the degradation of lignin into phenols. This simplified diagram shows what all the other authors want to imply: that lignin is broken down into its basic unit (coniferyl, sinapyl, or coumaryl alcohol). The basic unit is subject to oxidation followed by demethylation and converted to a phenol compound.

2.2.3.2 Microbial Phenols

Microorganisms are reported to also contribute to producing humic precursors. A great variety of phenolic and hydroxy aromatic acids are known to be formed by microorganisms from nonaromatic hydrocarbon substances. Many fungi, actinomycetes, and bacteria have been cited to be capable of synthesizing, by secondary metabolic processes, simple phenols and complexed polyphenols. However, such ability is deemed to be more a characteristic of fungi and actinomycetes than of bacteria (Stevenson, 1994). A variety of soil fungi, including *Aspergillus*, *Epicoccum*, *Hendersonula*, *Penicillium*, *Euratium*, and *Stachybotrys* species, have been reported to produce humic acid-like substances in cultures containing glucose, glucose-NaNO₃, asparagine, and peptone (Filip et al., 1974, 1976; Saiz-Jiminez et al., 1975). The substances formed are identified by chemical analysis to be composed of phenols, orsellinic,

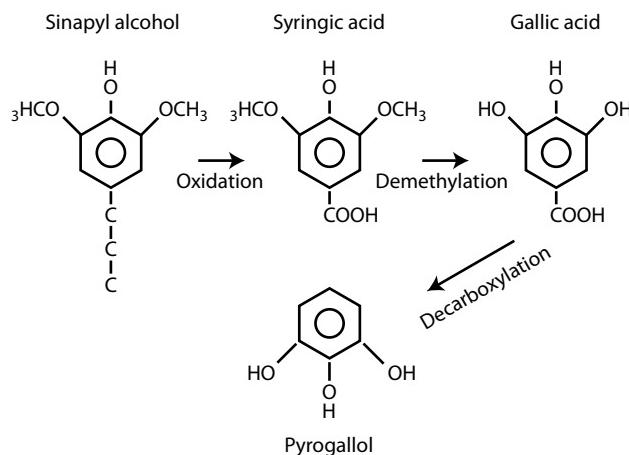


Figure 2.8 Simplified version of formation of pyrogallol by decomposition of lignin. (After Martin, J. P., and K. Haider, *Soil Sci.*, 111, 54, 1971; Martin, J. P., K. Haider, and E. Bondietti. Properties of model humic acids synthesized by phenoloxidase and autoxidation of phenols and other compounds formed by soil fungi. In: *Humic Substances. Their Structure and Function in the Biosphere*, D. Povoledo, and H. L. Golterman (eds.). Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Centre for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 71–86, 1975; Flraig, W., H. Beutelspacher, and E. Rietz. Chemical composition and physical properties of humic substances. In: *Soil Components, Vol. 1. Inorganic Components*, J. E. Gieseking (ed.). Springer-Verlag, New York, pp. 1–211, 1975; and Haider, K., J. P. Martin, Z. Filip, and E. Fustec-Mathon. Contribution of soil microbes to the formation of humic compounds. In: *Humic Substances. Their Structure and Function in the Biosphere*, D. Povoledo, and H. L. Goleman (eds.). Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Center for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 71–85, 1975.)

α -hydroxy-benzoic, β -hydroxy-cinnamic acids, anthraquinones, and melanins. Their appearance as dark-colored microbial products in the culture medium is the reason for associating them with humic acids, since phenols and their derivatives are known to be building constituents of humic matter. Formation of humic acid-like substances by mycorrhizal fungi has also been reported by Tan et al. (1978). A brownish substance is produced by the ectomycorrhiza *Pisolithus tinctorius*, grown in a Melins–Norkrans liquid culture with either sucrose or a mixture of L-malic and L-succinic acid as the carbon source. The brown-colored substance behaves similarly to fulvic and humic acid when subjected to extraction procedures with NaOH and HCl. The substance, which is soluble in base and insoluble in acid, exhibiting infrared absorption features similar to humic acid, is believed to be composed of uronic acids.

These acids are known to be waste products of microorganisms, and many authors are of the opinion that they contribute to the formation of humic matter (Flaig et al., 1975). The most probable mechanisms for the microbial synthesis of these humic precursors appear to be processes similar to those for the synthesis and/or decomposition of lignin. The two most probable mechanisms cited are the acetate–malonate and shikimic acid pathways. The data presented by Haider et al. (1975) suggest that in the acetate–malonate pathway, glucose is converted to orsellinic acid. Demethylation of the latter, followed by decarboxylation, yields resorcinol, a dihydroxyphenol. On the other hand, the shikimic acid pathway may produce pyrogallol as the end product. It is apparently a shorter pathway since gallic acid is reported to be formed directly by aromatization of shikimic acid, which by decarboxylation produces pyrogallol, a trihydroxyphenol. Both resorcinol and pyrogallol are prominent microbial phenols, or the phenols typically produced by microorganisms. Pyrogallol is also an important product in the synthesis and degradation of lignin, as discussed earlier. Polymerization of these simple phenols yields polyphenols. A simplified version of the formation of resorcinol and pyrogallol is illustrated in Figure 2.9. Although the two theoretical pathways

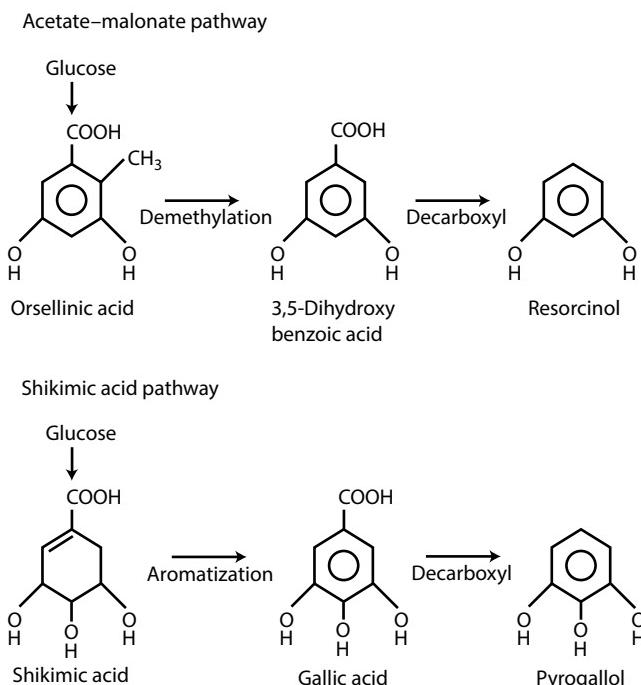


Figure 2.9 Bioformation of resorcinol and pyrogallol, according to the acetate–malonate and shikimic acid pathways, respectively.

have been designed to illustrate the formation of different intermediate products, often both mechanisms may end up yielding a similar phenol, e.g., pyrogallol, as the final product. It is only a simple matter of hydroxylation of resorcinol to convert it into pyrogallol.

2.2.3.3 Quinones

Quinones are hydrocarbon substances with the formula $C_6H_4O_2$. These compounds are usually yellowish to red, and biologically important as coenzymes, as hydrogen acceptors, and as key constituents of vitamins. They are derived from phenols and are derivatives of dihydrobenzene. Phenols formed by decomposition of lignin or by microbial syntheses are released in soils. They can be spontaneously oxidized in alkaline solutions, a reaction called *autooxidation* by Ziechmann (1994), and converted into quinones. The author indicates that the formation of quinone can be explained by the electron donor–acceptor theory. Ziechmann is of the opinion that the transformation is caused by *intermolecular electron transfer*, by which quinone is accepting 4- π -electrons donated by the phenol molecule. However, in a natural environment, enzymes are considered required in the oxidation of phenols. In this case, the transformation into quinones is not limited to oxidation of free phenols in soils but can also take place with phenol compounds within microbial tissue. The quinones formed can be secreted into the soil or can be released after the microbes die. Two groups of enzymes, phenolase and laccase, are considered to play an important role in the aerobic oxidation of phenols into quinones. Schubert (1965) reports that phenolase is capable of attacking mono- and dihydric phenols, whereas laccase catalyzes the oxidation of the polyhydric phenols. To illustrate the enzymatic oxidation of a phenol yielding a quinone, a simplified diagram of reactions involved is given in Figure 2.10. The orcinol in Figure 2.10 is formed from the decarboxylation of orsellinic acid, an acid produced in the acetate–malonate pathway as shown in Figure 2.9. Demethylation of orcinol yields catechol, which in the presence of a suitable enzyme, e.g., phenolase, will be oxidized and converted into O-quinone. It should be realized that this is not the only method for the formation of quinone, and many other methods are possible. For example, decarboxylation and oxidation of dihydroxybenzoic acid may also yield quinones (Flaig, 1966, 1988). A revised reaction, made by the present author to enhance comprehension, is given in Figure 2.10 for comparison with the oxidation reaction of orcinol and the electron donor–acceptor concept in the conversion of phenol into quinone.

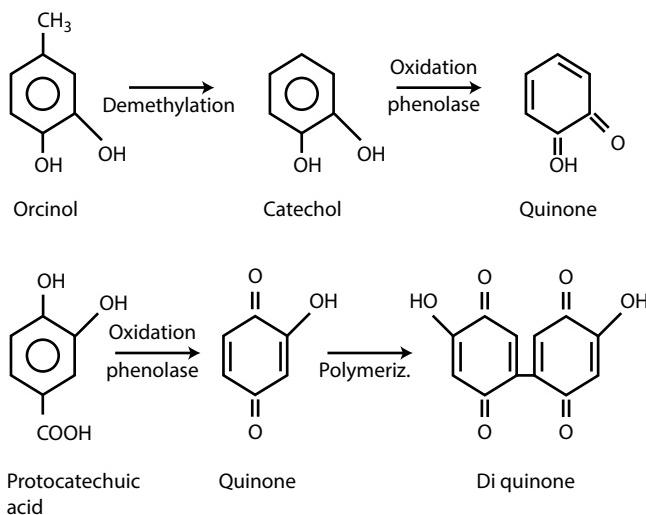


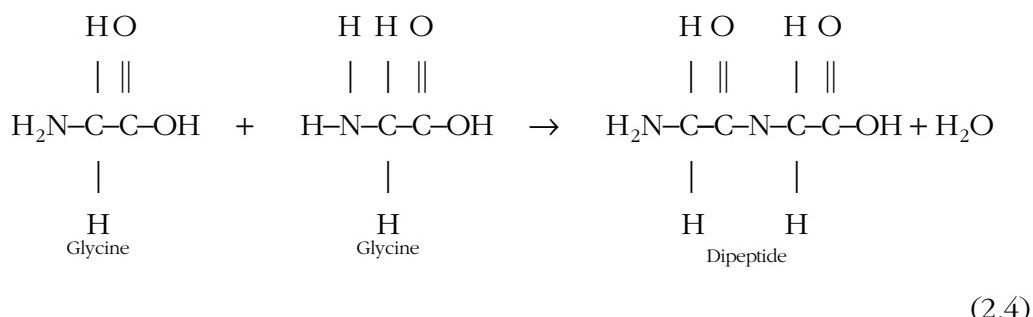
Figure 2.10 Simplified versions of catalytic oxidation of orcinol and proto-catechic (benzoic) acid, respectively, yielding quinone and diquinone. (After Schubert, W. J. *Lignin Biochemistry*. Academic Press, New York, 1965; Flraig, W., H. Beutelspacher, and E. Rietz. Chemical composition and physical properties of humic substances. In: *Soil Components, Vol. 1. Inorganic Components*, J. E. Gieseking (ed.). Springer-Verlag, New York, pp. 1–211, 1975; Stevenson, F. J. *Humus Chemistry. Genesis, Composition, Reactions*, Second Edition. John Wiley & Sons, New York, 1994.)

2.2.4 Protein and Amino Acids

In the early days, protein and amino acids were not considered compounds making up humic matter. Many scientists believed humic acid to be a plain hydrocarbon substance, and information has been presented off and on providing the argument for humic acid-like substances to be formed without protein. Even today, the idea still prevails that humic substances do not include peptides, nucleic acids, sugars, and fats (Hayes and Malcolm, 2001). These biomolecules are believed to be sorbed or coprecipitated at pH 1 or 2 during the isolation procedures. However, the majority today considers humic matter to be characterized by an elemental composition showing a nitrogen content ranging from 1% to 5%. The latter is assumed to be contributed by amino acids and/or protein compounds (Schnitzer and Khan, 1972; Stevenson, 1994), also called peptides, as will be discussed below. To people advancing the ligno-protein theory, protein and amino acids are considered important humic precursors (Kononova, 1961, 1966; Flaig et al., 1966). Some scientists even try to make a distinction between fulvic and humic acids on the basis of the types of amino acids present in their molecular structure.

Sowden et al. (1976) indicate that fulvic acids contain higher amounts of basic amino acids, whereas humic acids contain more of the acidic types of amino acids.

By definition, proteins are complex combinations of amino acids. These acids are given the name amino acids because the nitrogen in their molecules occurs as an amino (NH_2) group attached to the carbon chain. The acid part consists of a terminal C linked to an O atom and an OH group, often written as $-\text{COOH}$. The latter, called a carboxyl group, exhibits acidic properties, because the H of the OH radical can be dissociated. The protein is formed by the linkage of amino acid molecules through the carboxyl and amino groups:



The bond linking the two groups is called the *peptide bond*, and the compound formed is called a *peptide*, or protein.

Under refluxing with 6N HCl for 18–24 h, protein may be hydrolyzed into its constituent amino acids. Twenty-one amino acids are usually obtained as protein constituents; however, in natural environments, many other types of amino acids have been identified, which according to Stevenson (1994) do not belong to proteins. More than 100 amino acids and their derivatives are reported by Stevenson to be confined as constituents or products of soil microorganisms.

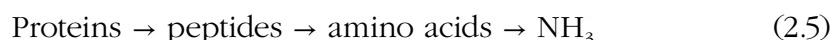
Both amino acids and protein are major sources of nitrogen compounds in soils. They are perhaps less difficult to break down than lignin, but more difficult than the carbohydrates. The ease of decomposition depends on the size and their molecular structures, which appear to increase in complexity with the type of compounds. The size and complexity in molecular structure increase from aliphatic, to aromatic, and heterocyclic amino acids. In addition, many of the proteins also occur in nature in complex combination, called *conjugated*, with other compounds, complicating further the decomposition of these compounds. For example, *glycoproteins* in plant

and animal tissue are proteins conjugated with glycogen. *Glucoprotein* is a protein present in combination with the carbohydrate glucose, whereas *lipoprotein* is protein conjugated with lipids. *Mucoprotein*, an important form of protein in the mucous layers of plants and animals, is supposed to be protein combined with uronic acids and other sugars. All of these factors will, of course, affect the rate of decomposition of protein and amino acids. For more details on the basics of amino acids and proteins, see Tan (1998, 2000).

2.2.4.1 Decomposition of Protein and Amino Acids

In contrast to lignin and phenols, protein and amino acids are major food sources for microorganisms. The nitrogen in these substances is an essential element for the growth of microorganisms as well as for the higher plants. Hence, it is expected that protein and amino acids will be subject to immediate attack by a host of microorganisms. These processes are part of the nitrogen cycle in soils and the environment. From the array of decomposition products produced, some will be adsorbed by clay minerals, whereas others will be used in the formation of humic substances. This part of the degraded protein and amino acid is considered temporarily resistant to further mineralization into NH_3 .

The main reaction process for the decomposition of protein and amino acids is hydrolysis. Hydrolysis of protein, brought about by the enzymes proteinase and peptidase of soil microorganisms, results in cleavage of the peptide bonds, releasing in this way the amino acid constituents. The latter substances are broken down further into NH_3 by the enzymes called *amino acid dehydrogenase* and *oxidase*. Schematically, the main pathway of decomposition can be illustrated as follows:



The decomposition reactions above involve processes called *deamination*, causing the destruction of the amino group or its conversion into NH_3 gas as part of the nitrogen cycle. Deamination can take place in aerobic as well as in anaerobic conditions, hence can be distinguished into *oxidative* and *non-oxidative deamination*, respectively (Gortner, 1949; Stevenson, 1986).

The reaction for oxidative deamination can be written as follows:



Anaerobic deamination may result in (1) deamination and reduction and (2) decarboxylation, as can be noticed from the reactions below

1. Deamination and reduction:



2. Decarboxylation:



Reaction 2.7 indicates that deamination is characterized by the destruction of the amino group and its transformation into ammonia, NH_3 , gas. In contrast, reaction 2.8 shows that decarboxylation involves the decomposition of the carboxyl, COOH , group into CO_2 , and the subsequent transformation of the amino acid into an amine compound. The enzyme required for decarboxylation, called *amino acid decarboxylase*, is produced by *Clostridium* bacteria. When formed in animal bodies, some of the amines produced are reported to have important physiological effects. For example, *histidine decarboxylase* in animal tissue can produce histamine, an amine that can stimulate allergic effects and/or gastric secretions. Another enzyme, *tyrosine decarboxylase*, is an intermediate in the formation of *adrenaline*, an amine functioning as a vasoconstrictor. It is usually released in the bloodstream when a person or animal is startled or frightened (Conn and Stumpf, 1967).

All of the proteinaceous substances in their slightly or highly degraded forms are considered by many scientists to play an important role in the formation of humic matter. Most of the nitrogen content in fulvic acid is contributed by amino acids, whereas at least one-half of the nitrogen content in humic acids can be accounted for by amino acids. Lower percentages of the nitrogen in humic acids are present as NH_3 , a compound apparently derived from the deamination reaction as shown in reaction 2.7. The nitrogen compounds associated with humic acids are assumed to be linked to the central core of the humic molecules.

2.2.5 Miscellaneous Nonhumic Substances in Humus

Other biochemical compounds of importance in the synthesis of humic matter present in soils are lipids, nucleic acids, chlorophyll, vitamins, and

hormones. Today, pesticides and their degradation products should also be added to this list in view of the increased influence of agricultural and industrial operations on the soil ecosystem.

2.2.5.1 Lipids

Owing to their amphiphilic character, lipids are one of the compounds that have been catapulted from miscellaneous to very prominent substances in structural chemistry of humic substances by the recent development of the *micellar* (Wershaw, 1986, 1999; von Wandruska, 1998) and *nanotube membrane* concepts (Tan, 2011) in humic acid formation. Micelles are believed to form by amphiphilics arranging their hydrophobic ends away from the surrounding water medium. These concepts will be discussed in more detail in later sections.

Lipids are heterogeneous compounds of fatty acids, waxes, resins, and oils. The term lipid does not imply a particular chemical structure, as with amino acid, but the name is used for substances that are soluble in fat solvents, such as ether, chloroform, or benzene. Chlorophyll and carotenoids are included in the group of lipids. For more details on the basics and types of lipids, reference is made to Tan (1998) and Stevenson (1994). Some of the soil lipids have found their origin from higher plants, such as waxes covering leaves and fruits. Also important sources of soil lipids are terpenoids contributed by Coniferaceae and Myrtaceae plants. However, many of the soil lipids may have been derived from microbial tissue. Bacterial cells contain 5% to 10% lipids, whereas fungi may contain 10% to 25%. The microbial products are the main sources of the glycerides and phosphatides in soil lipids.

Lipids are known to affect the physical properties of soils; however, not much information is available on this topic. They are hydrophobic compounds, and, therefore, will reduce the degree of wetting of soils. A high content of wax in soil humus is expected to make the soil water repellent, whereas high concentrations of stearic acids, a lipoidic compound, have been implicated in causing soil fatigue. Resistance to decomposition may vary considerably among the different types of lipoidic compounds. Many will decompose rapidly in well-drained soils, such as fatty acids, whereas others are relatively more resistant, such as wax, terpenoids, sterols, and paraffinic compounds. In general, it is assumed that in most soils, sufficient amounts of microorganisms are present that can attack even the strongest lipid. Soil microorganisms are even available that can disintegrate oil spills,

which are lipid-like compounds. The decomposition products may be in the form of lipoidic acids, which are high molecular weight organic acids containing hydroxyl and carboxyl functional groups, e.g., palmitic acid and stearic acid. These acids are important agents in the weathering of rocks and minerals, in the dissolution of plant nutrients, and in the mobilization and transportation of elements, critical in plant nutrition and soil genesis (Tan, 1986, 1998). Currently, lipids are also considered to contribute to the synthesis of the humic molecule. Paraffinic compounds have been discussed earlier as essential components of humin and kerogen (Hatcher et al., 1985).

2.2.5.2 Sterols and Steroids

These compounds, classified as *derived lipids*, have received increased attention as additional potential components of the humic molecule. The name sterol is derived from *stereos* (meaning solid in Greek) and *ol* from alcohol; hence, sterol means solid alcohol. The best-known sterol is *cholesterol*, which upon radiation will form vitamin D. These compounds are produced by animals and plants, as well as by microorganisms. *Phytosteroids* have been detected in fungi, *Chlorella*, *Chrysophycea*, *Diatomycea*, seaweed, and sphagnum. Soybean, rapeseed, spinach, cabbage, and palm trees are some of the plants known to contain steroids. In particular, the vegetation of peat and peat bogs is believed to be rich in steroids (Ziechmann, 1994; Gortner, 1949). Many of these sterols and steroids are known today to exhibit medicinal properties, and this is the reason why peat is often used for medicinal purposes. Peat baths were taken in the old days for therapy of gynecological and rheumatic diseases, and even today mud baths are offered in many European health spas and clinics. These therapeutic properties of peat, known for a long time, have currently been traced to humic acid as the dominant component of peat. Since the phytosteroids are present in soils as decomposition products of plant residues, chances are that they may participate in building up the humic molecule.

2.2.5.3 Nucleic Acids

Nucleic acids, first isolated in 1869 by F. Miescher from the nucleus of plants, are polymers of high molecular weights. Their repeating units are mononucleotides, rather than amino acids. Two types of nucleic acids are generally

recognized: (1) deoxyribonucleic acid (DNA) and (2) ribonucleic acid (RNA). Both DNA and RNA consist of long chains of alternating sugar and phosphate residues twisted in the form of a helix. The strands are bonded together by the purine bases adenine and guanine, and by the pyrimidine bases thymine and cytosine. For the basics of nucleic acids in soils, see Tan (1998).

Upon decomposition of plants, these nucleic acids will be released into the soil. Although they are one of the organic compounds from living cells that are apparently broken down rapidly in soils, they are also potentially capable of being incorporated in the humic molecules. As shown in Figure 2.11, the molecular structures of especially the nitrogenous bases lend themselves very well to rapid interaction with other humic precursors. Because of their nitrogen and phosphorus content, nucleic acids are expected to be important sources for soil nitrogen and phosphorus. Humic acid contains a considerable amount of nitrogen that cannot be accounted for in analysis. This unaccounted for nitrogen content, called *HUN* for *hydrolyzable unknown nitrogen* or simply *unknown nitrogen*, is believed to be associated with the nitrogen in nucleic acids or their derivatives (Schnitzer and Hindle, 1980).

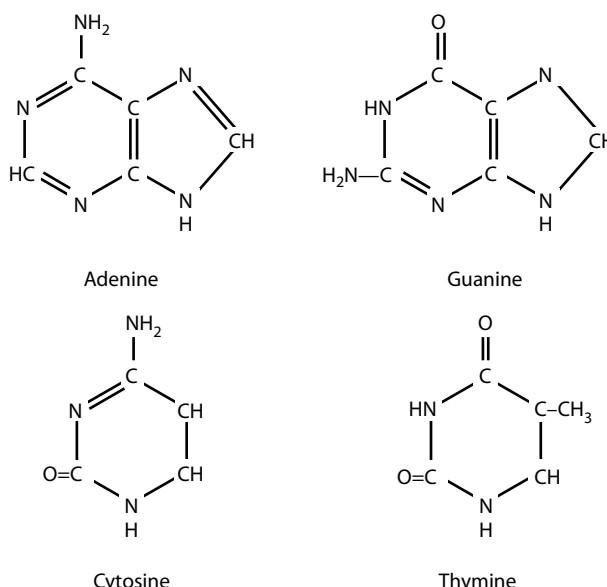


Figure 2.11 Molecular structures of nitrogenous bases of nucleic acids showing functional groups, potentially active for interactions with other humic precursors.

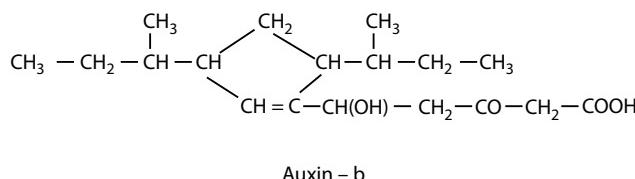
2.2.5.4 Growth-Promoting Substances

Humic matter has often been implicated in promoting hormone-like effects in plant growth. Stimulated seed germination, rapid root elongation, elongation of young seedlings, and accelerated shoot growth have frequently been assumed to be caused by a hormonal growth effect of humic matter (Poapst and Schnitzer, 1971; Poapst et al., 1970; Gumiński et al., 1977). Some truth may be present in these allegations since hormones can be released from plants and incorporated in the humic molecular structure. They are released into the soil upon plant and microbial decomposition. However, growth-promoting substances are not confined only to hormones since a number of other compounds are currently noted to possess similar effects as hormones. Degradation of lignin due to microbial decay is reported to produce substances exhibiting hormonal activity (Stevenson, 1994). Vitamins are also recognized to be able to promote plant growth. These growth-promoting substances in general are synthesized by plants and soil microorganisms. They are formed by the organisms for a specific purpose that up to now remains mostly a mystery. Although their concentrations are always reported to be very low, they are expected to be present in soil humus and are potential participants in building up a humic molecule. The extremely low concentrations are perhaps one of the reasons why in many cases the effect is rather obscured.

2.2.5.5 Plant Hormones

The term hormone is originally used for compounds affecting specific growth functions in animal bodies, and refers initially to chemical compounds secreted by the endocrine glands (Gortner, 1949). In plant science, it was Charles Darwin who started the idea of a substance present in plant tissue causing seedlings to bend when exposed to light. Darwin's theory, discussed in his book *Power of Movement in Plants* (1881), was later supported by results of light experiments in growing plant cells, conducted in 1910 and the following years by Boysen-Jensen (1936). Since then, many investigations have been carried out on the subject of growth-promoting substances in plants, which finally resulted in the term *hormone* being adopted for compounds showing the capacity to stimulate the growth of plants. The name *auxin*, from the Greek term meaning "to increase," was later suggested by F. Kögl for this group of plant regulatory compounds (Gortner, 1949).

Many types of natural and artificially produced hormones are known today, e.g., auxin-a, auxin-b, heteroauxin, and β -indoleacetic acid. Auxin-a



Auxin - b

Figure 2.12 Molecular structure of auxin showing carboxyl and hydroxyl functional groups for possible interactions with humic precursors.

and auxin-b can be isolated from the oil extracts of corn, mustard, sunflower, and flax plants. Human urine also contains substantial amounts of these types of auxins. Indoleacetic acids are artificially produced auxins. Substances regulating plant growth can also be produced by microorganisms. A fungus called *Gibberella fujikuroi* is noted to produce a compound named *gibberellin*, which in low concentrations promotes root growth. The artificially prepared product, called gibberellic acid, a crystalline acid with the formula C₁₉H₂₂O₆, is associated with and similar in effect to gibberellin. Another example is the hormone mentioned above under the name heteroauxin. It is reported to be formed by a variety of fungi, including yeast, and has been isolated from *Aspergillus niger* and *Rhizopus* sp. Heteroauxin is considered highly effective as a growth-promoting substance especially in lower plant life. This hormone has found today practical applications in industry and horticultural operations. Synthetic heteroauxins are, for example, β-indolebutyric acid and α-naphthalene acetic acid. They are presumably active in root formation and have been noted to induce formation of roots on plant cuttings, making possible the commercial propagation of plants that normally cannot be propagated by this method.

As indicated above, this group of compounds may be incorporated in the synthesis of the humic molecule and is then the reason for the alleged hormonal effect of humic matter. The molecular structure of auxin (Figure 2.12) shows the presence of carboxyl and hydroxyl groups for easy hook-up to phenols, quinones, lignin, and other humic acid precursors by chelation or other interaction processes.

2.2.5.6 Vitamins

These compounds have hitherto been considered only of importance in animal and human nutrition. The term *vitamine*, from which the present-day vitamin is derived, has been used to describe chemical substances essential

in the diet of animals and humans that exhibit a hormone-like or enzyme action affecting the control and coordination of specific reactions in the animal or human body (Gortner, 1949).

The vitamins are usually distinguished into the (1) fat-soluble vitamins, e.g., vitamin A, D, E, and K; and (2) water-soluble vitamins, e.g., vitamin C and vitamin B complex. At present, it is especially the vitamin B complex that has attracted much attention in plant physiology and humic acid chemistry. It is called the B complex since it is composed of several types of vitamin Bs, each responsible for specific functions in the plant body. Thiamin, riboflavin, nicotinic acid or niacin, vitamin B₆, biotin, and pantothenic acid are examples of vitamin B. This B complex also includes aminobenzoic acid and inositol.

Since these vitamins are produced by plants, they are suspected to also play an important role in plant life, although little is known on this aspect. The best-known vitamins produced by plants are perhaps vitamins A and C. Vitamin A is formed by carrots in the form of β-carotene, whereas vitamin C is produced especially by red pepper, citrus, and pineapple plants. It is also called *ascorbic acid* owing to its antiscorbutic effect. For commercial purposes, vitamin C is synthesized from glucose, which is why its molecular structure shows close similarities to a pentose or sugar (Gortner, 1949). It lacks a carboxyl group, but perhaps the OH groups (Figure 2.13), behaving as Arrhenius or Brønsted acids, are the reasons for considering it as an acid. Scientifically it is an acetone, since it is classified by Gortner (1949) as a 2,3-dienol-L-gulufuranol-acetone. The role of vitamin C as a redox agent or antioxidant in plant tissue is still under investigation.

In contrast, more is known on the effect of the vitamin B complex on plant life. For example, biotin and the related vitamin H, essential for normal skin growth in humans, are believed to affect the growth and respiration of *Rhizobium trifolia* and other microorganisms. Amino-benzoic acid, another vitamin B, is noted to stimulate bacterial growth. Thiamin is known to be produced by higher plants and microorganisms for use in their metabolism. In higher plants, thiamin is concentrated in seeds, and especially in the embryos. Nicotine acid, also called niacin, is found in cereals, fruits, and vegetables. Of course, meat, milk, and egg are the primary sources of niacin. It is believed to be converted into nicotine, an alkaloid present in tobacco plants, by oxidation and methylation (Gortner, 1949).

As is the case with hormones, these vitamins may also be included in the synthesis of humic matter and are parts of the reasons for the growth-stimulating effect shown by humic substances. The molecular structures shown for some of the vitamins (Figure 2.13) suggest again the presence of

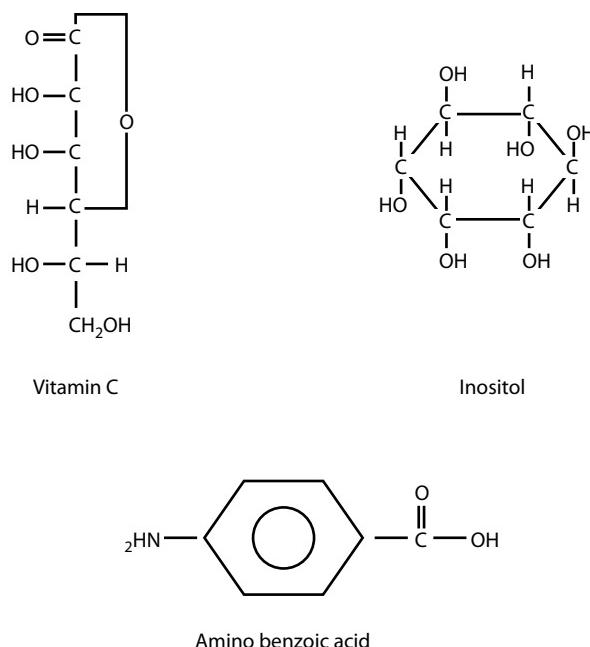


Figure 2.13 Molecular structures of vitamin C, inositol, and aminobenzoic acid, showing functional groups, potentially active for interactions with other humic precursors.

chemically active functional groups for easy hook-up to phenols and other humic precursors.

2.2.5.7 Xenobiotics

The name xenobiotic refers to foreign organic substances, here meaning foreign to the soil. They are introduced into soils by the modern and extensive agricultural and industrial operations of today. The major xenobiotics expected to participate in the synthesis of humic matter include pesticides and their degradation products, e.g., the triazines, substituted ureas, and phenylcarbamates. These substances have the capability of forming stable complexes with soil organic constituents, which greatly affect their chemical behavior in soils in several ways. Such interactions may increase their persistence in soils, bringing with it a variety of effects on the environment as discussed earlier by Tan (2000). The behavior of the pesticide incorporated in the humic molecule may go one way or the other. It may remain chemically active and toxic, or it may often lose its identity and behavior as a pesticide. The latter process is called biodetoxification by Tan (2000). Biodegradation of pesticides is reported

to yield chemically active substances that can link with carbonyl, carboxyl, phenolic-OH, and amino groups of other soil organic substances to form humic acid-like compounds. The redox property of humic acids, as discussed before, can in turn play a definite role in the transformation of pesticides. For example, s-triazine is reported to be converted into its cationic form by humic acid, and the protonation process causing the development of the positive charge occurs on the ring-nitrogen atom (Weber, 1970), as illustrated in Figure 2.14. This process can also be explained by the electron donor–acceptor concept (Ziechmann, 1994). The triazines are considered the electron donors, whereas humic acids are the electron acceptors. A positively charged triazine is produced when an electron can be transferred from the electron-rich triazine to an electron-deficient quinone in the humic molecule. The transfer also results in humic acid becoming negatively charged.

Of interest perhaps is also the chemical compound 2,4-D, which in low concentrations can function as a growth-promoting substance, but at relatively higher concentrations will act as a herbicide. The formal use of 2,4-D today is as a weed killer. However, in the past, it has found application in controlling blooming and fruiting of crops. It is also used in rubber (*Hevea brasiliensis*) cultivation to increase the latex production of the trees. The structure of 2,4-D (Figure 2.15) shows an active carboxyl group, whereas the

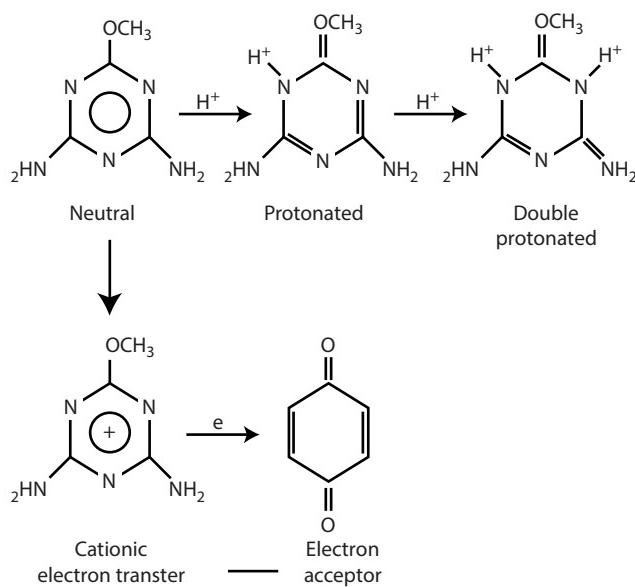


Figure 2.14 Schematic illustration of the transformation of a neutral triazine molecule into a cation by induced protonation and by the electron donor–acceptor concept.

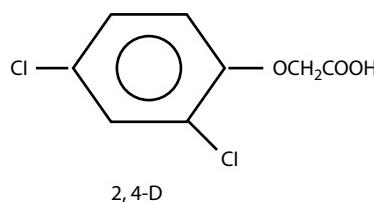


Figure 2.15 Molecular structure of 2,4-D showing the presence of Cl and a carboxyl group providing locations for potential reactions in the synthesis of humic matter.

chlorine on the aromatic ring can be easily replaced by organic substances, including humic acids. By incorporation into a humic acid molecule, the chemical activity of 2,4-D can be preserved. It can remain active as a weed killer and this is reflected by a process called *residue effect*, or it may contribute to the hormone-like behavior of humic acid. Since in all likelihood, only extremely small concentrations are chelated in the humic molecule, the hormonal effect is more likely to prevail over the herbicidal effect. However, it is also possible that these activities can be erased, since as an integral part of the humic structure the identity of 2,4-D has faded completely. Persistence of this compound in soils is usually not a big issue since it is known to exhibit only a short half-life or mean residence time.

2.3 Humic Fraction of Humus

The purpose of this section is to examine the alkali-soluble fraction of humus, which was believed to be the true *soil humus*. The extraction of the black amorphous material called humus was apparently intended to discover and identify the nature of its components. When the results turned out that the extracts were—and are still today—materials what we now call humic substances, many tended to think or conclude that humus was nothing more than humic compounds. This alkali extract or the humified fraction makes up the bulk of soil humus, whereas the nonhumified fraction is usually present in relatively smaller amounts (Tan, 1998; Stevenson, 1994). The humified compounds are the most active substances in soils, possessing electrical charges and exchange capacities exceeding those of the clay minerals. They are essentially new products in soils synthesized from the nonhumified materials released during the decomposition of plant and animal residue with or without the assistance of microorganisms. Like clay, humified

materials are also major building constituents of soils and the process of their formation is called *humification*. The name *humic material* was used by Christman and Gjessing (1983) to identify this humified fraction of soil humus. However, the present author prefers to use the term *humic matter*, a name used earlier by Page (1930), as discussed before. It is applied by the present author in analogy to the term *organic matter*. Like organic matter being a mixture of a great number of organic components, so is humic matter composed of a variety of humified substances, e.g., humic acid, humatmelanic acid, and fulvic acid.

2.3.1 Issue of Humus Acid and Huminsäure

In the German literature, humic matter is called *humussäure* or often also *huminsäure* (Döbereiner, 1822; Scharpenseel, 1966), whereas in the Russian literature it is known as *humus acid* (Orlov, 1985). Unfortunately, these names are misunderstood as misconceptions or misnomers, hence ignored by most of the scientists in the Western Hemisphere. However, the present author is of the opinion that it is in fact a unique way to unequivocally distinguish humus from its soluble (humic) fraction. By creating and using the name humus acid, scientists in Eastern Europe, long considered the cradle of humic acid research, have perhaps wittingly or unwittingly presented a proper delineation between humus and its alkali-soluble fraction. By naming the latter humus acid or *huminsäure*, because of its relation to the acidic substances in the extracts, the term should be viewed as an umbrella term and, as erroneously stated by most Western scientists, is not just another name for humic acid. Humic acid is one component of humus acid or *huminsäure*, as are fulvic acid, humin, humatmelanic acid, brown and gray humic acids, and others in the humic matter family being the other important components. In the more modern Russian literature, humus is also treated as a different entity from humic matter, with the latter also accepted and grouped as one of the components making up humus (Bogoslovskiy and Levinskiy, 2006).

2.3.2 Early Approaches of Investigations

In the early period of investigations to study the humic fraction by extraction of soil humus, two lines of approaches can perhaps be noted. One of these is called by the current author the *analytical* or *chemical approach* because the approach is based on extraction using alkaline and/or organic reagents, and

analyzing the chemistry of the isolated products. It is perhaps the most common method that appears to be the most popular or the most followed. A second line of investigations is called the *functional approach* by the author, which does not resort only to extraction of soil humus and isolation of its humic fraction but also examines soil humus *in situ* as affected by differences of vegetation cover, land use, and/or by the environment in general. The *function of humus* in plant growth and its role in the soil form the basis in this second approach, which makes this method stand out from the analytical or chemical approach. This second approach is, therefore, not only more complex but also depends on a great deal of personal judgment and practical skill of the investigator, which can perhaps be considered less straightforward than chemical analyses. The two approaches seem to have vague boundaries since overlaps and cross-overs are very common and our pioneers in humus science were noted not to hesitate about using one or the other whenever it suits their purpose. To avoid confusion and misunderstanding, the author wishes to make it clear that throughout the following sections, the text uses the term humus for the humic fraction, because this was the name used by the early scientists for the alkali-soluble fraction of humus.

2.3.2.1 Analytical or Chemical Approach

To identify the chemical substrate that can be called truly humus, the fully decomposed, rather uniform, brown to blackish often spongy or somewhat jelly-like and amorphous “rotten” plant material is subjected to alkali extraction. Achard (1786) was reported to be the first scientist to extract peat in his study of humus, but it was De Saussure (1804) who was given credit for introducing the name humus in soil science for the extracted products. Since then, this method of extraction of “humus” has attracted a lot of research attention from scientists both in the United States and in Europe. As noted earlier, lignin was identified as a dominant component that resulted in Waksman (1938), a renowned U.S. scientist, to name the extracted product *lignin humus*, in other words *lignin humic matter*. However, it was especially in Germany where the earliest tangible discoveries were made. On the basis of origin or source, Sprengel (1826) succeeded in isolating humus fractions he called (1) *acid humus*, extracted from peat and other types of “acidic” vegetation, and (2) *mild humus*, which has been isolated from soils under deciduous hardwood vegetation. As noted by Sprengel, the acid type of humus is generally more stable to decomposition than the mild humus. By redissolving the acid humus isolates in sodium carbonate solutions and

subjecting them to further purification, an ash-free black substance was obtained by Sprengel that in dry conditions exhibited a shiny black metallic luster. It tended to dissolve slightly in water and upon electrolysis traveled to the positive pole, indicating that it was electronegatively charged. All the above features suggest that what in the old days was called "humus" is in fact by today's standards humic substances. This became even more evident when in 1938 Springer reported that the alkali-soluble humus could be divided into brown and gray humic acids. The gray humic acid was in Springer's opinion comparable to Sprengel's mild humus. By publishing an article with the title "The biochemistry of humus formation," Whitehead and Tinsley (1963) tend to support the old concept of humus being identical to humic matter. As suggested by their analytical details and the text of the paper, old entrenched ideas are very hard to change, and unfortunately considering humus to be humic acid seems to be perpetuated until more recent times. However, one important achievement during this early period is the development of the now standard NaOH extraction procedure, as indicated earlier, as the accepted method for isolating humic and fulvic acids. A flow sheet of the older procedure, adapted by the International Humic Substances Society (IHSS) and the Soil Science Society of America for their more modern versions, was published in 1950 by Russell and Russell.

In the early efforts with the NaOH extraction method, difficulties in completely extracting all the humic components have raised some concerns. Among the several reasons for failure in obtaining complete extraction of the humic components is the idea that humic substances are strongly bonded by the inorganic soil particles, especially sesquioxidic clays. The sodium pyrophosphate, $\text{Na}_4\text{P}_2\text{O}_7$, methods were then developed with the expectation to increase the ease of extraction by the phosphate chelating the Al, Fe, and other sesquioxidic metals (Kononova, 1961). Another reason for incomplete extraction of humic substances is perhaps the presence of differences in degree or rate of decomposition that has apparently escaped research attention. The author tends to believe that the organic substances released from plant cells are not all together at once transformed into humic compounds. Some of the nonhumic components available for humification may perhaps have been drastically changed into humic molecules, but many may only be partly transformed, whereas several others are perhaps only slightly or peripherally affected, and so on. In other words, individual organic components may show different degrees or rates of decomposition, which when sorted out properly, their transformation may appear as a continuum. Such a condition may perhaps create the variations noted in the

nature of humic substances isolated by subsequent extractions. Although it is not a huge problem that deserves full and immediate attention, it is perhaps worth further research efforts. A more critical issue is the disturbing findings that the extraction may have changed the nature of the humic compounds (Bremner, 1950), which have started a series of activities to solve the issues above that have lasted until late in the twentieth century. Extraction procedures under a nitrogen atmosphere were developed to avoid autoxidation and/or alkaline hydrolysis during the processes, which became for some time the most acceptable procedures. Nevertheless, many scientists seemed to remain skeptical, and this was perhaps the reason for the introduction of the *operational concept* of humic substances in 1985. It was presented by geologists and hydrologists of the U.S. Geological Survey and adopted by chemists and geochemists (Aiken et al., 1985; Gaffney et al., 1996a), apparently as an answer to the controversial issue of producing artifacts during extraction. The isolated compounds were not believed to be the natural humic compounds, but were allegedly formed or changed during the operational procedures, hence—perhaps as a compromise—are called operational compounds. However, for many soil scientists, it is a concept too controversial to accept summarily (Schnitzer, 1982a). It has also left humic acid research by many soil scientists in disarray for lack of justifying scientific investigations on allegedly “fake” substances. The suggestion presented by Hayes and Malcolm (2001) for conducting scientific research of fake compounds out of curiosity is perhaps allowed at federal institutions, such as the U.S. Geological Survey, but at many universities in the United States it is too farfetched and practically unjustifiable.

2.3.2.2 Functional Approach

As indicated above, in this approach attempts have been made to study humus on the basis of its function in plant growth and of the role humus plays in the soil rather than analyzing the nature of its chemistry. This concept was applied early in the twentieth century by German scientists (Scheffer and Ulrich, 1960) to distinguish humus accordingly on its alleged role in soils into *dauerhumus* (resistant or stable humus) and *nährhumus* (nutrient humus). As the name implies, *dauerhumus* is relatively stable and decomposes slowly in the soil. It is believed to be the humus fraction insoluble in organic solvents, and in particular acetyl-bromide used by Scheffer and Ulrich above. On the other hand, *nährhumus* can be used as a plant nutrient and is reported to correspond to the humus fraction

soluble in H_2SO_4 (80%) solutions. It is believed to be composed of carbohydrates and proteins and allegedly will decompose readily (Russell and Russell, 1950). The above discoveries are most probably used by Stevenson (1994) as the basis for developing his idea to partition soil organic matter in two major groups, e.g., (1) *stable humus*, a term that is suspiciously coined from *dauer* humus and (2) *labile (active or light) humus* that is in the line of *nähr* humus because of its high nutrient content and function in nutrient cycling.

In forestry, it is common to recognize *mor* and *mull* humus, names introduced by Müller (1878) in Denmark for differentiating the types of humus formed under different types of forest vegetation. It is generally noted that *mor* humus occurs under a coniferous forest or a heath vegetation; hence, this type of humus is perhaps comparable with Sprengel's acid humus. The concept of *mor* humus includes an undecomposed layer, lying on top of an F-layer ($F = förmultningskikt$, German for fermentation), underlain by a somewhat structureless dark layer, called H-layer ($H = \text{humus}$ or German *humusämneskikt*), equivalent to the *sapric layer* in U.S. Soil Taxonomy. The undecomposed top layer is comparable to the fraction referred to earlier as litter. On the other hand, *mull* humus is formed more under a deciduous hardwood forest, and hence, contains more bases, especially Ca, than *mor*. It is perhaps comparable to Sprengel's mild humus and generally believed to be affected by soil organisms, especially earthworms, in its formation. In *mor* humus, the fungi are credited for the decomposition of the raw material (Russell and Russell, 1950).

By today's scientific standards, *mor* humus is normally associated with spodosols (podzols) under especially coniferous vegetation. The soil usually lacks an A horizon and the *mor* humus layer often lies directly on the E horizon, and this is most probably the pedon originally classified by Russian soil scientists as "podzols" (Orlov 1985; Russell and Russell, 1950). In contrast, *mull* humus is usually found in soils, called brown earths or brown forest soils in the old days, which today are classified as alfisols in U.S. Soil Taxonomy (Soil Survey Staff, 1990, 2006). In view of its "mild" nature due to the presence of sufficient amounts of Ca, humus in mollisols may perhaps also qualify to be called *mull* humus.

2.3.3 Concept of Humus toward the Third Millennium

The concept of humus used during this modern period still pertains to the decomposed part of the dead organic fraction in which, as defined, the

structure of the original material has disappeared completely. As also discussed earlier, in U.S. Soil Taxonomy, today humus is referred to as sapric material, from the Greek term *sapros*, meaning rotten, and hence is the most highly decomposed organic fraction. Sapric materials are commonly dark gray to black, which soil taxonomists believe will change very little physically and chemically with time in comparison with the fibric and hemic fractions (Soil Survey Staff, 1990). From all the discussions in the above sections, it can perhaps be concluded that humus is not equivalent to humic substances, but to be defined as humus, the material must contain, in addition to humic compounds, also the “raw” (nonhumic) organic components released by decomposition from plant tissues and/or other organisms.

Two major categories of humus are also recognized by the current author: (1) terrestrial humus, characterized by high lignin contents and its decomposition products, and (2) aquatic humus, characterized more by the presence of carbohydrates and its decomposition products. The alkali-soluble fractions of each of the two groups above consist then of humic substances that may differ accordingly. The chemistry of the terrestrial humic compounds is expected to be characterized by lignin, whereas that of the aquatic humic fraction is believed to be more carbohydrate in nature. Indications are that most soil scientists seem to agree with such a perception (Steinberg, 2003). However, new disturbing ideas have surfaced because of sudden flare-ups of controversies from the use of the term SOM (soil organic matter) for humus by some scientists. As defined in Section 2.1, humus is part of, but not equal to, SOM, which is, by definition, the total organic matter content in soils. By using the term SOM and humus interchangeably or indiscriminately as done by Schnitzer (2000) and Wershaw (2004), one would not know whether the author is referring to the total soil organic matter content or just to that part of the soil organic matter called humus. To support his use of SOM, Schnitzer points to Stevenson's (1994) definition of humus as a synonym of SOM. This is not only perpetuating an old—now invalidated—idea, but it is a misconception, eroding the new concepts and definitions reached due to advancing techniques and science in organic matter, as related in the sections above and in Chapter 1. Notwithstanding the controversial definitions in his table 2.3, special efforts have been made, in fact, by Stevenson (1998) to distinguish in his book “humus” from “SOM” in the section on modern-day concepts of humus. However, his references to and usage of SOM in the text are indeed very difficult to read, hence to some may be confusing and misleading. Perhaps his use of acronyms may also be a factor for some of the confusion. The issue of the use, overuse, and/or misuse of acronyms in

the identifications of the various fractions and/or types of organic matter has been addressed in Section 1.6 of the current book.

2.4 Issue of Glomalin

Lately, researchers at the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) made the discovery that challenges the concept and importance of soil organic matter and/or humus. In 1996, Sarah Wright reported the presence of *glomalin* in soils as a glycoprotein produced by arbuscular mycorrhizal fungi. Scientists from the USDA-ARS are jubilantly claiming that its benefit as a carbon storage place far exceeds that of soil humus and/or humic acids and necessitates a reexamination of the concepts of humus and/or soil organic matter (Comis, 2002). Glomalin was also praised, if not glorified, as the “superglue” of the century, extremely beneficial in the development of soil structures. This resulted in a number of scientists scrambling to consider the reevaluation of fungal contribution to the formation of soil organic matter and in increasing soil particle aggregation (Magdoff and Weil, 2004; Haddad and Sarkar, 2003). However, only mixed information in support, if any, of glomalin is available from other sources in the literature, whereas the article by Comis (2002) seems to be in fact a form of a promotional report only from the USDA-ARS Information Office. Most of the information published since then in popular magazines, e.g., *Green Power Conferences*, and local news media are either repeats of the Comis paper above or excerpts thereof in the form of a public relations effort of the USDA-ARS to increase awareness of the U.S. public to the discovery of glomalin. A follow-up scientific and very interesting paper is, however, presented by Nichols and Wright in 2005. The material, published as part of the senior author’s PhD dissertation at the University of Nebraska, Lincoln, Nebraska, provides a credible effort in comparing glomalin and humic acid in several mollisols and ultisols of the United States. Unfortunately, extraction of humic acids from soil samples that have been treated first with Na-citrate solutions for glomalin extraction is subject to many arguments and casts doubt of yielding unaltered humic compounds. Autoclaving and heating at 121°C are expected to break down the humic molecules, hence should be avoided if natural humic acids are to be obtained by the method of the IHHS as indicated. If the true nature of the extracted humic product could have been determined or confirmed by the authors above by either infrared (IR) absorption analyses or scanning electron microscopy (SEM), the

controversies as stated above would have perhaps been avoided. Examples of IR spectra and SEM micrographs of several humic substances are available for comparison in the literature (Schnitzer, 1965a; Stevenson and Goh, 1971; Chen and Schnitzer, 1976; MacCarthy and Rice, 1985; Tan, 2005).

The compound in question, called glomalin, is produced by the mycorrhizae fungi and detaches from the hyphae when the latter moves into the soil and becomes a component of soil organic matter (Haddad and Sarkar, 2003). Reports have surfaced lately that glomalin as such is allegedly still unknown and as a specific protein molecule has yet to be isolated. It is allegedly an operational compound, named by Canadian scientists as *glomalin-related soil protein* or *GRSP*, because its occurrence is defined by the method of its extraction and the reaction with an antibody, Mab32B11 (Rillig, 2004). Nichols and Wright (2005) also made reference in their journal article that what they have isolated was in fact glomalin-related soil protein. The compound is reported to be found in abundance only where the particular mycorrhizal fungus is abundant, and it is speculated that it allocates carbon to the fungus (Treseder and Turner, 2007). The latter raises questions as to its origin as a true fungal metabolic product. It is also reported that glomalin is not produced by “noninfected” plant roots (Magdoff and Weil, 2004), while it must be realized that plant roots are major contributors of soil organic matter, especially in mollisols, the “grassland soils” of the Great Plains. Moreover, with a reported carbon content of 30% to 40%, glomalin is second only to humic acid’s 45% to 57% carbon content in effectiveness of organic carbon sequestration (Tan, 2009). This is supported by Nichols and Wright (2006), who presented a mean carbon content (%C) of 28% to 43% in their glomalin-like extract versus 49% carbon in their humic acid-like product. Of interest is that Hayes and Clapp (2001) placed glomalin in the nonhumified group of humus. Many also believe soil humus to be the most persistent pool of soil organic carbon. Because soil humus possesses a mean residence time of several hundred years, Fiorentino et al. (2006) indicate it to be the major sink of soil organic carbon, hence controlling carbon dioxide emission to the atmosphere. Therefore, pending future information, glomalin as a glycoprotein can perhaps be considered at the present stage of knowledge, if not as an operational compound, as one important type among the many others of the total soil protein fraction that makes up the soil humus.

Chapter 3

Concepts of Humic Matter

3.1 Overview: From Biopolymers to Nanotube Supramolecular Assemblages

At the end of Chapter 2, humic matter was addressed as an integral part of soil humus. It was the purpose then to explain it as the alkali-soluble fraction of soil humus, and resolve the controversy that the extracted substances were identical to soil humus, which was the general idea held in the past. This chapter will discuss the concepts of humic matter as a chemical compound or as a mixture of several types of humic substances with different chemical compositions.

The intention is to focus now on the idea or general understanding of what, in fact, humic matter is all about, or what it is when one is talking about humic acid. Since it was isolated more than a century ago for the first time from soil humus and named *humic acid*, its identity and concept were surrounded by a cloud of controversy and debates, which have not abated even with our present knowledge in humic acid science. Although the understanding of this organic substance has changed with time, slowly at the start and somewhat abruptly toward the end of the twentieth century, no satisfactory agreement has been reached about a concept that is not controversial and truly representative of the compound called humic acid, or better *humic matter*. The change from one concept to another was not in a sense of a sequential evolutionary process; in other words, the new concept is not built from the older one, but it was created more because of the lingering opposing views. Today, several concepts on humic matter are available and it depends on the particular concept what one is talking about when referring to “humic acid.” Traditionally, humic substances have been considered as either natural or artificial compounds produced during extraction (Aiken et al., 1985; Stevenson, 1994; Frimmel et al., 2002; Tan, 2003a).

Since humic acids were isolated by Berzelius in 1839, most people believed them to be natural substances. The numerous data collected since then from a great number of investigations tend to suggest that humic substances are polymeric in nature. This *biopolymer concept* became so popular that the concept was embraced by most scientists for almost two centuries.

Criticisms and objections raised at that time on the humic acid concepts mentioned above are not directed specifically to the compounds being polymers, but are leveled more to the nature of the extracted humic compounds. They are viewed by some as substances that are allegedly created during the extraction procedures (Bremner, 1950), as discussed at the end of Chapter 2. The *operational concept*, introduced in 1985 by geologists and hydrologists (Aiken et al., 1985), is thought perhaps to provide a scientific basis for the idea of humic substances being fake compounds or compounds artificially produced during isolation analyses. However, it was a severe blow to the concept considering them to be natural compounds. Fortunately, the widespread occurrence of humic substances and their importance in agriculture, industry, and especially in natural ecosystems have reversed the operational concept at the turn of the century into the original idea of natural compounds. During the period of the operational concept, the science of aquatic organic matter was advanced and organic substances given the name DOM (dissolved organic matter) and the like have gained in importance. The issue was discussed in some detail in Chapter 1. This rapid development of aquatic humic matter science is perhaps also a contributing factor toward recognizing terrestrial humic substances as natural compounds. Humic substances are now considered important components of natural organic matter (NOM) in terrestrial and aquatic ecosystems. This concept has refueled the enthusiasm for renewed investigations on humic matter in soil science. Humic acid research by soil scientists has buoyed back from its forced hibernation-like conditions, as evidenced by the number of publications of several new concepts published in rapid succession by the end of the twentieth century, challenging the established polymer concept. Wershaw (1992, 1999) suggested the micellar concept, which was supported by von Wandruska (1998). This was soon followed by the supramolecular association concept of humic compounds published by Piccolo (2002) and Piccolo et al. (2003) and the nanotube concept thereafter by Tan (2011).

3.1.1 In Vivo Humification Hypothesis

To make this overview complete, the following controversial issue is added for contemplation by scholars, professionals, authorities, and investigators in

humic acid research. Lately, some soil scientists have expressed a very startling idea that deviates from the normal concept of synthesis of humic matter in soils by claiming that humic substances are in fact already produced in the plant body (Susic, 2008; Bogoslovskiy and Levinskiy, 2006). It is called “*in vivo* plant biosynthesis of humic substances” as cited by Paciolla et al. (1998) from the literature, but renamed “*in vivo* humification” in this book. Unbelievably as it sounds with the current scientific knowledge, the hypothesis is increasingly making headway because of the indisputable fact that all the elements for formation—lignin, amino acids, protein, carbohydrates, and the needed chemical reactions for a humification process—are present in live plant cells. Hence, assembling the biocomponents into humic matter, indeed, does not require a soil environment. In the author’s opinion, polymerization, aggregation, self-assembling, or whatever chemical processes are involved, can take place or are taking place already in the plant body, even more easily than in soils. For example, during the growth of plants, protein is produced by assembling the amino acid components. Another example is cellulose by assembling the monosaccharides, whereas this cellulose is later lignified when needed as plants become older, and in particular in woody plants. Considering all of the above, plants should then be capable of combining lignin, protein, carbohydrates, and other metabolites into prototypes of humic compounds or *proto-humic substances*. The present author believes that this term is more appropriate and perhaps more acceptable than the name humic acid and the like to critics and opponents. Aggregation or self-assembling of the components into prototype humic substances is possible, especially during the senescence state or when plants reawaken in spring time, because during those periods the substances are believed to be mostly present as monomers. The idea above is, of course, not immune from the usual criticisms, and disagreements have been presented by several authorities in humic acids. One of the most recent objections is offered by Piccolo et al. (2003), who believe that humic substances cannot be the products of cellular synthesis as are the other biopolymers, but rather the products of cell death. The issue is perhaps that such a concept has been created before its time but it is nevertheless worthwhile to pursue it through further research.

The different concepts of humic matter developed over time as discussed above will be discussed in somewhat more detail below. It depends on the readers, professionals, scientists, and investigators which one to believe or accept. Except for the operational concept, which is today practically obsolete, each of the other concepts has its own merits and all are useful today at least among proponents of the idea.

3.2 Biopolymer Concept

This is the oldest concept of humic matter and considers humic substances to be polymeric compounds. By definition, a *polymer* is a large compound composed of many repeating smaller structural units called *monomers*. The term polymer is derived from the Greek words *poly* (= many) and *meros* (= parts). Polymers can be distinguished into (1) *natural polymers*, such as nucleic acids, proteins, and natural rubber, and (2) *synthetic polymers*, such as nylon, polystyrene, silicone, and synthetic rubber. The process of linking the monomers together to form a polymer is called *polymerization*. For example, a lignin polymer is built of several or a great number of monomeric lignin units, as illustrated in Figure 2.7. Another example is polysaccharide, which is made up of repeating monosaccharides bonded together by *glucosidic bonds* and so are proteins that are formed by amino acids connected together by peptide bonds. When two monomers are connected together, a dimer is formed (Figure 2.2), and when three monomers are linked up, the compound is a trimer, and so on. As indicated above, the process of formation of polymers is scientifically known as polymerization, but sometimes the term *condensation* is also used. Scientifically, the polymerization process in today's polymer chemistry can be distinguished into two groups. The first group is called *condensation polymerization* in which the chemical reactions between the monomers are called *condensation reactions*. This type of polymerization is characterized by the loss of a molecule, usually H₂O or water, as can be noticed in the lignification process (Figure 2.7). This is the type of polymerization that commonly occurs in the synthesis of humic matter. The second group is known as *addition polymerization* by which the monomers are linked together without losing an atom or a molecule. Due to covalent, strong C–C and C–H bonds, addition polymers are generally chemically inert and nonbiodegradable, features that are in contrast to those exhibited by condensation polymers. The latter are biodegradable, which accounts for humic substances to be subject to decomposition processes, hence are not that stable for long periods as postulated by many scientists (Russell and Russell, 1950; Whitehead and Tinsley, 1963). The mean residence time (MRT) of the humic fraction is estimated to be 250 to 1900 years (Stevenson, 1994), which means that it has only a short lifetime in terms of geologic time periods.

Polymers of lignin alone or polysaccharides only do not make up humic compounds. It takes both lignin and protein as one chemical unit, serving as the smallest repeating units that will build a humic compound according to the ligno–protein theory. The humic molecule formed is called a biopolymer

because the monomeric units of lignin and protein are derived from biological tissues. In similar fashion, polysaccharides in combination with protein units, serving together as the smallest chemical units repeating themselves in a molecule, will create aquatic humic substances. In crystal chemistry, such repeating units are called *unit cells*. Unit cells are applied in building the framework of clay minerals in soils (Tan, 2011). Although Kononova (1961) was cited by Piccolo et al. (2003) to have introduced the polymeric concept of humic matter, such a polymeric view has in fact been known for some time. It was allegedly introduced as early as 1835 by Jöns Jacob Berzelius, after his historical analyses in extracting humic substances. Berzelius' original definition may, of course, differ from the modern-day definition of polymers (Berzelius, 1839). Later, Waksman (1932, 1938) referred to humic matter—soil humus was the term used at that time—as large molecular associations of lignin and protein compounds. Perhaps, it is Flaig (1975), in Germany, who has truly defined humic matter to be organic polymers from lignin degradation products in his efforts to promote his well-known ligno–protein theory. Other prominent scientists from the German school, influential in supporting the existence of humic matter in the form of chemical polymers, are Scharpenseel (1966) and Flaig and Beutelspacher (1951). In Canada, Schnitzer (1976) suggested humic matter to be benzene carboxylic acid polymers, whereas in the United States, Stevenson (1994) wrote in his book about his well-known phenol or quinone dimer theory of humic substances. Today, Swift (1999) is a strong supporter of this polymer concept, whereas Piccolo (2002) has voiced strong reservations in favor of his supramolecular theory.

Although it was lignin and its degradation products that first caught most of the attention, the ensuing research at that time by other scientists has also revealed the importance of carbohydrate polymers as key components of humic matter, especially of fulvic acids. As shown in Figure 2.5, phenol or aromatic units can be created from fructose, a sugar that upon condensation can form polymers. This has laid the foundation for the carbohydrate concept of humic acids, which at the start of the new millennium has gained acceptance with the rapid development of theories on aquatic organic matter and its humic matter fraction.

The biopolymer concept above—advancing the idea of condensing small molecules into large biopolymers of humic substances—seems to provide a workable hypothesis. It is, among other things, not running afoul with the carbon and nitrogen composition of humic substances. The latter are always reported within relatively consistent ranges, which is perhaps caused by the humic polymer assuming an elemental composition not much different

from that of the constituent monomers. The elemental composition of the monomers may then more likely be reflected in that of the humic polymers. Consistency in C_{org} and N content is supported by the following facts. Organic matter is assumed to contain, on average, 58% C_{org}; hence, in elemental analyses, most scientists use this as a constant for conversion of C_{org} into SOM. The conversion factor, called the *van Bemmelen factor*, equals then 100/58 = 1.724 (Tan, 2005). The relatively consistent nitrogen content is also due to the proteins exhibiting more or less a constant nitrogen content of 16%, an important factor for the observed consistent humic nitrogen contents. Soil chemists are also used to applying this constant in determining the soil's protein content by multiplying the soil's %N found with a factor 6.25 (= 100/16). Another supporting factor is that the principles in basic polymer chemistry indicate that the polymer would acquire a molecular weight equaling the sum of molecular weights of the participating monomers, minus the moles of H₂O lost during the condensation reactions (Holleman, 1946). The formation of sucrose, a disaccharide, by condensation polymerization of the monomers, glucose and fructose, serves as an illustration:

Polymerization



However, in the case of polymerization of a huge number, involving different varieties, of monomers—producing heteropolymers—the molecular weight can become indeed unknown. This then accounts for the traditional concern in unpredictability and/or variations obtained in molecular weight determinations of humic acids. The latter is one of the major factors considered by a group of scientists for challenging the validity of the polymer concept as addressed above. The new theories—the micelle, supramolecular, and nanotube concepts—suggested to replace it, will be discussed in more detail below.

3.3 Operational Concept

The issue of artifacts appears to have started early during the birth of the science of humic substances. It has increased since then in seriousness to become a major concept during the previous century as reflected in the

operational concept created by the founders of the International Humic Substances Society (IHSS) in 1985. Criticisms were launched in the early days at the reality of *crenic* and *apocrenic acids*—old names for *fulvic acids*—isolated by Berzelius (1839) and Mulder (1862). Opponents argued that they were oxidized products of humic substances, and disputed the correctness of the chemical composition (Hermann, 1845; Kononova, 1966). With the accumulation of data over the years, more and more people seem to resist considering laboratory products similar to natural compounds. In the beginning of the twentieth century, the concept that humic substances are not natural compounds at all but are merely substances formed by the extraction procedures seems to be firmly adopted by a group of authorities in organic matter science (Waksman, 1938; Bremner, 1950). This concept implies that they are in essence fake compounds or as stated above are not natural but artificially produced substances. They are also assumed to be heterogeneous in composition (Felbeck, 1965) and refractory in nature; hence, they cannot be placed into a definite class of chemical compounds as commonly executed in the classification of substances into chemical categories, such as polysaccharides, proteins, amino acids, and lignin (Gaffney et al., 1996b). Because of this presumption, the notion is that humic substances do not exhibit clear molecular structures. As summarized by Clapp and Hayes (1997), they are gross mixtures of macromolecules, and two humic molecules in any batch will likely be dissimilar in nature to each other. Aiken et al. (1985) and Hayes et al. (1989) also indicate that these organics have not been formed biologically for performing specific biochemical functions, hence cannot be translated into specific functional terms.

Since to a large number of people the concept of humic acids has also been vague and confusing, especially during the period 1970–1980, the problem seems to be exacerbated by an apparent “identity crisis” on what exactly humic matter is. It attracted at that time relatively little interest, and the few soil scientists and organic chemists struggling with the problem faced opposition from fellow scientists, peers, and supervisors, who considered humic substances as “dirt” hardly worth studying. Such resentments became so unproductive for advancing humic acid science that Schnitzer (1982a) felt compelled to take action for a change in attitude from skepticism into optimism at the 1982 International Congress of Soil Science in New Delhi, India. The title of his article—“*Quo vadis* organic matter research?”—reflects the prevailing mood of despair among many soil scientists, and basically is a plea for better reasoning and a more positive view in dealing with settling disagreements on humic acid issues. The recently

founded IHSS had evidently contributed to providing more exposure to humic substances in the quest for some basic legitimacy of their presence. The major scientists responsible for the establishment of this scientific society were R.L. Malcolm and coworkers from the U.S. Geologic Survey, Water Division, Denver, Colorado, and M.H.B. Hayes of the Chemistry Department, University of Birmingham, England. At its first meeting held in 1985 at Estes Park, Colorado, several of the society scientists opted for the use of the term *operational compounds* for these substances, which reflects their formation due to specific analytical operations (Aiken et al., 1985). The concept was accepted and used as a working hypothesis by members of the American Chemical Society in their humic acid research (Gaffney et al., 1996a). As a scientific association, the IHSS is also considered by scientists, students, professionals, and the general public as the authority in humic matter. Hence, it is no wonder that the idea of artificially produced substances has resonated as the magic concept worldwide, causing a negative view on humic substances. This concept has not changed with the dawn of the new century, since the *Proceedings of the Symposium of the International Humic Substances Society*, published in 2001 (Clapp et al., 2001), seems to use and enforce the idea of humic substances being operational compounds. Although this approach is perhaps an attempt to provide a scientific basis for working with fake or artificially produced humic substances, such a grand intention seems to be misunderstood. The implication that it provides some legitimate justification for further continued studies of fake humic substances is apparently not shared by most soil scientists. The general belief is that it is, in essence, only sidestepping the controversial issue of artifacts.

All of the above raise questions about why such great importance should be given to fake substances, or whether the current technology is incapable of identifying them properly. The statement issued by Hayes and Malcolm (2001), founding officers of the IHSS, in their most recent paper that it can be justified on the basis of scientific curiosity and the advancement of scientific awareness raises more questions than answers. Research efforts solely for scientific curiosity and raising awareness of fake substances are difficult to justify and unproductive for scientists at research institutions and universities, especially where the motto “publish or perish” prevails. Fortunately, the ubiquity of humic matter in terrestrial and aquatic ecosystems, and its overwhelming applications in agriculture and industry, as indicated before, seem compelling enough for the IHSS to later reassess its operational concept. This was soon followed by the IHSS issuing a statement on its website that “humic substances are major components of natural organic matter (NOM)

in soil and water as well as in geological deposits".* The author assumes that strong evidence obtained from research by those few loyal to the idea of humic substances being real (natural) compounds may have contributed to the above reversal in concept by the IHSS. However, this is another story that will be addressed in the next section.

3.4 Issue of Real Compounds

With the advances in humic acid chemistry during the last two decades of the twentieth century, another concept of humic substances, completely opposite to the one discussed above, seems to surface. Some are vocal about it, whereas most remain more discreet. Although many of the proponents have not stated it specifically, their efforts to present especially a molecular structure show their convictions about humic substances being real compounds with definite compositions. As indicated by Hayes et al. (1989), a molecular structure as defined by a discrete elemental composition can be constructed only for a real compound.

The concept, considering humic substances as real compounds in nature, is not new in fact and finds support apparently from a greater number of people than expected as noticed from the following discussion. It originated perhaps with Achard (1786) and Berzelius (1839) when they started to study humic substances. In addition to these pioneers, Mulder (1840, 1862) believed that humic substances were definite chemical compounds characterized by a specific composition. Although the initial idea of assessing humic substances as real compounds was challenged several times, as discussed in the previous section, the concept seemed to continue to simmer over the years, especially in Russia and Europe, the birthplace of humus and humic acids. It flared up again at the beginning of the twentieth century when new evidence presented by Oden (1914, 1919) and others provided the right fuel. Kononova (1966), who wrote a skeptical review about the early fights, was not exactly refuting the idea but was more concerned about and in disagreement with the elemental composition. In Kononova's opinion, humic substances must contain nitrogen, as can be noticed in the hypothetical structure advanced by the author for humic acid. Orlov (1985) is more straightforward, indicating that the reality of humus acids must be accepted for many reasons. They are present in soils as well as in aquatic

* <http://www.humicsubstances.org/whatarehs.html>, accessed January 30, 2014.

environments and in marine and lacustrine sediments. He indicates that they can be isolated from natural waters often without using the usual procedures employing alkaline and acidic reagents. In his opinion, spectral characteristics are not only reproducible, but the spectral features are also identical between extracted and nonextracted humus acids in whole soils. Among the German scientists, it is perhaps Flraig (1975) whose studies are based on the concept of formation of humic substances as really being chemical compounds in nature, characterized by specific molecular structures. He notes that the changing nature of their chemical composition is due to the dynamic or transient nature of humus—Flraig's term for humic substances—because of the never-ending decomposition and new formation processes in nature. However, far more noteworthy in this respect is Ziechmann (1994), who states that "humic substances are natural compounds existing in reality." In his opinion, humic substances exhibit specific characteristics that can be measured and that distinguish them clearly from other soil organic compounds, e.g., acetic acid. Consequently, they are a separate group of compounds with a composition of their own. He believes that by accepting the division of humic substances into humic acid and fulvic acid, the presence of a specific chemical composition is implied. An alternative concept is proposed by Ziechmann in dealing with the debate on chemical composition, which will be discussed in a separate section below. For the reasons discussed above and in view of the increased application of humic substances in other fields, e.g., technology, industry, pharmacy, and medicine, Ziechmann questions the merits in defending any further the operational definition. In the United States, it is Stevenson (1994) who views humic substances as distinctive soil components, different from the biopolymers of plants and microorganisms, although his statement that the term humic substances should be regarded as a "generic name" is confusing the issue. However, Stevenson's opinion, that humic substances can be placed into a chemical category different from carbohydrates, protein, amino acids, and the rest of the nonhumified substances, infers that humic substances possess a definite chemical composition. Stevenson's dimer concept, revealing the molecular structure of humic substances to be composed of two monomers, is an extension of such a perception and supports the concept of humic substances to be real compounds. This perception reinforces the opinion of Ziechmann (1994) as discussed above. Another prominent scientist in the Western Hemisphere indicating discreetly that humic substances are natural organic products on the earth's surface is Schnitzer (1975; Schnitzer and Khan, 1972). His efforts on constructing molecular structures show

his convictions about humic substances being discrete natural compounds (Schnitzer, 1994). Together with Schulten, Schnitzer has presented several concepts on the molecular structure of humic acid assembled from products found in their degradation analyses (Schulten and Schnitzer, 1993, 1995). Such an assessment of Schnitzer's view is in agreement with the opinion of Hayes et al. (1989) on molecular structures as the property of real compounds, as discussed earlier. However, it is perhaps the work of Schulten (2001, 2002) that has provided a severe blow to the "artifact concept." Schulten's brilliant presentation of molecular structures of humic substances appears to indicate that structure—to proponents—is no longer an issue. However, to many others, these are only *pseudostructures* and/or *molecular modeling* of humic matter structures. Although they are indeed just structural models showing only molecular components thought to be present in humic substances, the fact is that a range of well-established and tested chemical units have been detected and are available to build a humic molecular structure. The presence of such structures seems to be indisputable, and strongly suggests the presence of real compounds. Therefore, considering humic substances as structureless artificial compounds created by the analytical extraction procedures may in the long run become very difficult to defend.

3.4.1 Controversy of Chemical Composition

As discussed in the preceding section, the concept of humic substances to be real compounds has been challenged over time, due to their alleged lack of a definite chemical composition. The term *chemical composition* can refer to the elemental composition, the functional group composition, and the many compounds considered "building blocks" that make up the molecule of the humic substance, although according to Hayes and Malcolm (2001), humic substances do not contain peptides, sugars, nucleic acid, and the like. For humic substances to be considered discrete chemical compounds, like sugars and nucleic acids, the authors claim that they should exhibit a chemical composition, expressed in a formula, suggesting their molecular weight. The following discussion will examine further the elemental composition, and issues on the other types of composition of humic matter will be discussed elsewhere in the appropriate sections of this book.

In the aforementioned sections, the controversy was presented on the problem of a characteristic composition, with one group claiming there is none, and other sides insisting that humic substances exhibit a definite chemical composition that distinguishes them from other organic

compounds. The first group maintains that the refractory nature allows for the isolation of humic fractions with a variety of compositions. None of these chemical compositions are characteristic for the humic substances, since the skeptics believe that any composition can be obtained, depending on the isolation and fractionation procedures. Among the staunchest proponents in this group are Hayes et al. (1989), who claim that humic substances cannot have unique molecular weights. The elemental composition, considered by these authors the most fundamental property, can only be exhibited by a discrete chemical compound. It is their firm belief that humic substances, being composed of complex nonstoichiometric mixtures, cannot be represented by empirical formulas, since their elemental compositions are only the average values of large clusters of molecules. In Stevenson's (1994) opinion, such an idea is unfortunate for teaching and learning purposes. The use of a simple structural unit would be advantageous in explaining the properties and reactions of humic compounds to students. Ziechmann (1994) declares it as a "dead-end street," which leaves investigators with no alternatives. He suggests abandoning further searches on chemical composition when carried out with the preconception of the absence of a molecular structure. In Ziechmann's opinion, this is part of the reason why no progress has been achieved or ever will be obtained in the future.

On the other hand, the group that believes in the presence of a chemical composition has yet to determine the composition characterizing the humic substances, which up until now has allegedly eluded all efforts, although chemical formulas for humic substances have been presented as early as in 1839. Formulas such as $C_{24}H_6O_{12}$ and $C_{24}H_{12}O_{16}$ were presented by Berzelius (1839) for his crenic and apocrenic acids, the fulvic acids of today. More recently, a chemical formula of $C_{308}H_{328}O_{90}N_5$ has been reported by Schnitzer (1994) for a humic acid molecule. Two years later, Schulten (1996) claimed to have determined, by computer modeling, monomers of humic acid with a composition of $C_{308}H_{335}O_{90}N_5$, which is almost the same as Schnitzer's formula. Schulten's formula shows the humic acid monomer to correspond with a molecular weight of 5478 and a nitrogen content of 1.28%. That such a composition may apply to artificially prepared materials is without doubt; however, whether it really characterizes a humic acid molecule is still subject to argument. This will be discussed in more detail in Chapter 8 on chemical composition of humic matter.

According to several scientists, the refractory nature of humic substances, showing an apparently endless variation in composition, is probably more the result of their natural formation in soils. As mentioned earlier, they are transient organic components in soils (Flaig, 1975; Ziechmann, 1994),

forming a dynamic system that decomposes and is formed again continuously, changing their composition in the process. The problem becomes even more complex because of the many reactions and interactions, adding and losing organic constituents during the humification process.

Consequently, the nonhumified compounds and humic substances in all phases of formation and degradation make up the humus mixture in the soil, which is sampled and extracted for humic substances. This accounts for the extreme difficulties in the identification of a characteristic monomer of a humic substance, e.g., humic acid. Which of the substances isolated should then be considered to exhibit the characteristic composition of humic acid? They are all real organics formed in nature and the extracted preparations, labeled operational compounds or artifacts, are nevertheless noted to be reproducible in behavior and properties (Orlov, 1985). The infrared absorption spectra are reproducible and the carbon and nitrogen contents are determined to be relatively constant. The issue is perhaps created only by a disagreement among scientists on what to call humic substances. A good analogy can perhaps be given with protein, which can be extracted from soils and plants. Everybody agrees that they are discrete substances, built from a variety of amino acids and other organic compounds. Carbohydrates and nucleic acids may participate in their formation as noted by the presence of glucoproteins and nucleoproteins in nature. Therefore, they exhibit a multitude of formulas with different compositions since their formulation depends on the various types of amino acids and other components, and their configuration in the protein molecule. This is also true with humic acid since the data presented thus far underscore the humic molecule to contain phenolic or benzene, amino acid, and carbohydrate components (Flaig, 1988; Schnitzer, 1986, 1994; Christman et al., 1989). The controversy on chemical composition can perhaps be eliminated by accepting the various compositions noted in the so-called operational humic substances as the result of a multitude of interactions and arrangements of the different types of components, much in the same way as protein molecules are formed. The different phases caused by the humification and decomposition processes, although important, may pose only minor problems, since most of the components are known today, with each exhibiting a discrete empirical formula. An example of how to apply an analytical chemical composition of a humic substance was presented by Steelink (1985), who used atomic ratios, as suggested earlier by Van Krevelen (1963) and Visser (1983), in the determination of a formula. Another system was presented by Orlov (1985), who claimed that the molecular weight of humic substances could be calculated from

their mean elemental composition. In this concept, any humic fragment or phase, containing a residue of amino acid, is assumed to have a minimum molecular weight, which is obtained by conversion of the total nitrogen content to one atom of nitrogen in the formula. More details about Steelink's and Orlov's concepts will be provided in Chapter 8.

3.5 Micellar Concept

The micellar model of humic matter was, in fact, introduced by Wershaw (1986, 1999) and supported by von Wandruska in 1998. *Micelles* are, by definition, submicroscopic aggregates of amphiphilic molecules in colloidal systems; however, in clay mineralogy, the silica tetrahedron and aluminum octahedron layers are also called micelles or *clay micelles*. *Amphiphiles* are compounds demonstrating both hydrophilic (water-loving) and hydrophobic (water-hating) characteristics in one molecule (Tan, 2011). Detergents and surfactants are industrial amphiphiles, whereas plant lipids are natural amphiphilic compounds. The name is derived from the Greek words *amphis* (= both) and *philia* (= love). The hydrophilic end (part or region), called the head of the molecule, is typically arranged on the micelle surface facing the surrounding solution (Figure 3.1). They are ready to attract water or interact with the surrounding water. These heads, normally polar, are composed of ions (charged particles), nonionic particles (noncharged), or zwitterions. The remainder of the molecule behind the "head" is usually visualized as the "tail" of the amphiphilic molecule. This tail is the hydrophobic part, and is normally arranged in the center (or inside) of the micelle, away from the surrounding solution, as the name "hydrophobic" implies it rejects water. This tail is nonpolar and contains the aromatic and aliphatic components. The process of formation of such a structure is called *micellization* and the micelle formed can assume different shapes (or forms) and sizes, varying from single spheres or globules to cylinders and bilayers. The bilayers are called *membranes*. Most micelles, composed of simple ionic amphiphiles, are usually spherical and rather homogeneous in size with a radius equaling the length of the hydrocarbon chain of the amphiphilic components (Figure 3.1).

The micelle or micellar concept described above is applied in the construction of humic molecule structures by Wershaw (1986). Wershaw claims humic substances to exhibit molecular structures in the form of micelles formed through spontaneous aggregation of amphiphiles. Although many plant biopolymers, e.g., phospholipids and amino acids, are already

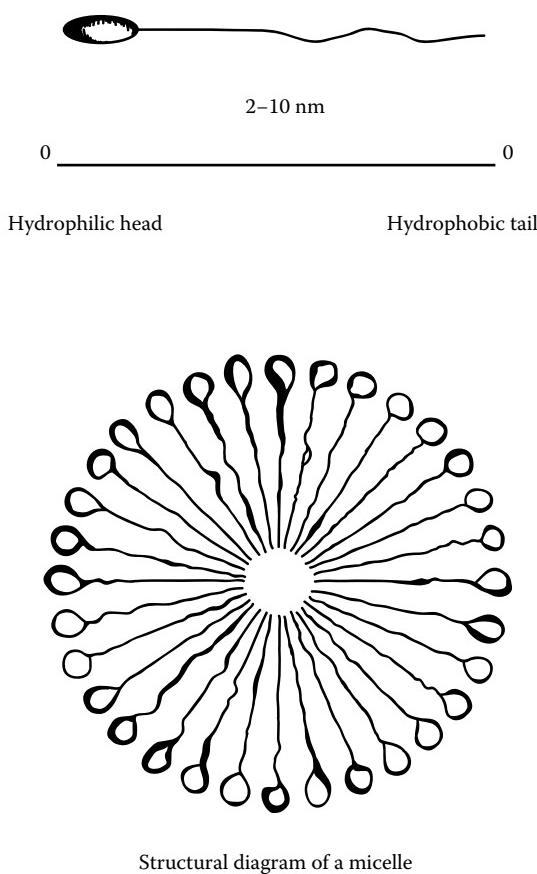


Figure 3.1 Top: Schematic of hydrocarbon chain of an amphiphile. Bottom: Structural diagram of a spherical micelle composed of single ionic amphiphiles, with a radius of $\pm 2\text{--}10\text{ nm}$. (Hand drawings by author; adapted from <http://en.wikipedia.org/wiki/File:Micelle.png>; accessed January 30, 2014.)

amphiphilic in nature, Wershaw believes that amphiphilic particles can be formed by depolymerization and oxidation of the lipid and aromatic plant components. Depolymerization of these biopolymers will break them down into unaltered smaller pieces, and further oxidation of one end or several parts of these smaller segments creates terminal carboxyl (COOH) groups. Hence, molecules are allegedly produced with hydrophilic (polar) parts on one end and hydrophobic (nonpolar) regions on the other end of the molecule. The polar hydrophilic heads consist then of the carboxylic acids, which are arranged on the outside or surface of the micelle facing the solution. The nonpolar hydrophobic ends (called tails), composed of mostly the

broken, unaltered polymer pieces, are consequently arranged accordingly in the inner sides of the micelle. At very low concentration, these amphiphiles exist mostly as monomeric particles, but will assume globular aggregates—the micelles—at concentrations greater than the critical micelle concentration (CMC). In natural waters, the DOC content is usually substantially lower than the CMC value; hence, Wershaw notes the difficulties in formation of micelle-like structures in aquatic or dissolved humic substances. On mineral surfaces, the humic molecular structures assume membrane-like characteristics. Three types of humic molecules, different in composition and behavior, are allegedly formed, and Wershaw also believes that three types of bonding mechanisms are involved in holding the amphiphiles together in the micelle. They are hydrogen bonding, π -bonding, or in Wershaw's words, "stacking of planar- π -donor–planar- π -acceptor groups to form a complex," and charge transfer complexation. To these, van der Waals and hydrophobic bonding should perhaps be added. Because they are all weak bonds, the micelle is expected to disaggregate or disperse easily into smaller aggregates or eventually into its different components. Indications of such a dispersion have allegedly been reported by Piccolo et al. (1996), who claimed that monocarboxylic, dicarboxylic, and tricarboxylic acids were capable of dispersing the larger humic micelles into smaller segments as a function of concentration of added acid and pH. The important implication following the observations above is that the dispersed broken segments are capable of reforming micelles; therefore, micellization and disaggregation may then continue on and on. This accounts for the common complaints of humic acids yielding upon extraction a confusing array of humic particles, different in sizes and shapes.

3.5.1 Spontaneous Aggregation and Disaggregation as Natural Processes

In natural soil ecosystems, such spontaneous aggregation and disaggregation processes are more common than many people would have expected. They are not isolated processes occurring only in micellization of humic substances, but they also take place on a macro scale in the formation of soil structures involving soil organic and inorganic particles. Crumb, granular, platy, and blocky soil structures are formed by processes known in basic soil science also as aggregation processes through a similar natural self-assembling mechanism. Because of this, the present author suggests to call such a spontaneous aggregation process in soil structure formation also a *self-assembling process* and the aggregates are then *self-assemblages* of soil structural units. The

self-assemblages of soil aggregates, composed of the four major soil constituents sand, silt, clay, and SOM, are then the *soil structures*. When the self-assembling processes are induced by cultivation, the structural units are also known as “clods,” but when they are formed in nature they are often called soil “peds” (Tan, 2009). Granular and crumb structures—the best soil structural units for agriculture—are created by such a spontaneous aggregation of sand, silt, clay, and organic matter. Continuous soil cultivation in crop production tends to destroy the soil structures above into single-grained structures. However, when after cultivation the soil is left alone in nature for some time, the soil particles are noted to “regroup” spontaneously back into the original structural types of either crumb or granular structures, depending on the soil organic matter content. The common bonds playing important roles in the aggregation or assembling processes are water bridging, hydrogen bonding, and metal bridging. Liming promotes formation of Ca and/or Mg bridges between the soil particles favoring the formation of crumb and granular structures. In natural ecosystems, Al and Fe ions are effective in creating these bridges. Spontaneous assembling, disassembling, and reassembling processes are especially unique in paddy soils, where the surface soil is puddled and made structureless for lowland rice cultivation. The author always notice that when the paddy soil, after the rice harvest, is left uncultivated under weed, the surface soil attains back its granular or crumb structure.

As a final note on the issue, the author wishes to draw attention also to the term *self-organization* often used interchangeably with *spontaneous self-aggregation* and *self-assembly*. The process of self-organization has some similarities with self-aggregation and self-assembly, and the differences are perhaps only a matter of semantics, interpretations, and/or formulating definitions. However, some people believe that self-organization is a nonequilibrium process, whereas self-assembly leads toward equilibrium and requires the components to remain unchanged during the reaction (Whitesides and Boncheva, 2002; Halley and Winkler, 2008). The author wishes to refrain from rendering judgment on the controversy and leaves the decision up to the readers and other more qualified authorities in these respective subjects.

3.6 Supramolecular Concept

The supramolecular theory is a new concept in the field of chemistry that became of importance in the second part of the twentieth century because

of its contribution in the studies of the structures of protein and DNA. The principles based on intermolecular forces were discovered in 1873 by Johannes Diderik van der Waals. However, this new concept was propelled to its current status under the name of *supramolecular chemistry* only after D.J. Cram, Jean-Marie Lehn, and C.J. Pedersen were awarded the Nobel Prize in 1987 for their contribution in this area (Lehn, 1993, 1995). Supramolecular chemistry considers chemical systems, called *supramolecular assemblies*, as multicomponent systems, assembled from atoms, ions, and/or discrete numbers of assembled molecular units. Such assemblies are supposed to form naturally without guidance or laboratory manipulations; hence, they are usually also known as molecular self-assemblies. They can be distinguished into intermolecular and intramolecular self-assemblies. In ordinary language, the formation process is called simply a spontaneous self-aggregation or self-organization process. The sizes of molecular assemblies are in the range of nanometers (nm) to micrometers (μm). Micelles and membranes are among some of the large assemblies. Here lies then the close relation and/or overlap between the micellar and supramolecular concepts. In contrast to traditional chemistry based on strong covalent bonding, the supramolecular concept is based on weak intermolecular bonds, such as van der Waals forces, hydrogen bonding, hydrophobic forces, electrostatic attraction, metal bridging, and $\pi-\pi$ interactions. Therefore, supramolecular compounds are considerably less stable than “conventional” molecular substances and tend to break apart easily, especially at high temperatures and at increased acidity or lower pH values. During the self-assembly process, some of the “raw” materials may aggregate into undesirable molecular associations. However, because of the weak intermolecular bonds, the chemically “wrong” aggregates are easily disassembled and the components rapidly reassembled to form the most stable association. The most stable assembly is one with the most thermodynamically stable arrangement of the constituent components. This ease of spontaneous assembling, disassembling, and reassembling processes to yield a thermodynamic stable molecular unit is considered a key feature of supramolecular chemistry in contrast with conventional chemistry.

The supramolecular theory discussed above was applied by Piccolo (2002) in humic acid chemistry to replace the polymer concept. It provides a fascinating new idea in humic acid chemistry, and a great number of scientists tacitly agree and have received the new concept with subdued criticisms (Sutton and Sposito, 2005). Several of the humic acid scientists have also expressed a wait-and-see attitude. This is perhaps more likely to be the result of a so-called *polymer concept fatigue*, as many are hoping somewhat anxiously for the appearance of new ideas that can solve the controversy of

molecular weight and many other issues in humic acid chemistry. Although it is indeed an excellent concept, the supramolecular theory presented by Piccolo has in fact created several new questions. Among Piccolo's many objections to humic polymers, published repeatedly several times in international and local journals, his major concern of importance—the issue of molecular weights—will be discussed here. To avoid being repetitious, interested readers are referred to Piccolo's original publications for his other objections (Piccolo et al., 2001, 2003; Piccolo, 2002).

3.6.1 Issue of Molecular Weights

The molecular weight of humic substances is perhaps one of the most controversial issues and the prime reason for many scientists criticizing the well-established polymer concept on humic substances. The theory that has lasted for two decades has allegedly failed to provide for a commonly accepted molecular weight of humic substances. Molecular weights of humic substances are noted to vary widely from a few thousand to 10,000,000 or more. Flaig (1958) reported values in the range of 30,000 to 50,000 for humic acids and 10,000 for fulvic acids. On the other hand, molecular weight values of only 1000 to 2000 have been reported by Schnitzer and Khan (1972). However, in basic polymer chemistry, such a wide variation in molecular weights is not uncommon. The range can be from the one hundreds for molecular weights of dimers to the millions for huge polymers. Some believe that the range from low to high molecular weight values may look like a continuum (Stevenson, 1994; Piccolo et al., 2003). An analogy to the issue is perhaps the monosaccharides polymerizing into huge heteropolysaccharides, where the molecular weights may vary from a few hundred in the dimer sucrose to more than a few millions in the polymer dextran.

The alleged failure to find a molecular weight for humic substances acceptable by all is one reason for Piccolo to attempt turning humic acid science from the well-established polymer concept into a supramolecular concept. The wide range in molecular weights allegedly reported in the literature, from 500 to 1,360,000 Da, a variation Piccolo believes too large for any of those values to be the characteristic mass of humic substances, is the reason for him to abandon the idea of humic compounds being polymers. The dalton unit (Da), as used by Piccolo and many other scientists for molecular weight, is very confusing. This unit is usually defined as follows:

$$1 \text{ Da} = 1 \text{ amu} = 1/12 \text{ of } {}^{12}\text{C} = 1.66 \times 10^{-24} \text{ g}$$

in which amu = atomic mass unit (Tan, 2011). Molecular weight values, ranging from 500 to 1,360,000 Da, as cited by Piccolo, will then assume the following values, respectively:

$$500 \text{ Da} = 5 \times 10^2 \times 1.66 \times 10^{-24} \text{ g} = 8.3 \times 10^{-22} \text{ g}$$

$$1.36 \text{ million Da} = 1.36 \times 10^6 \times 1.66 \times 10^{-24} \text{ g} = 2.3 \times 10^{-18} \text{ g}$$

These are practically inconceivably small values for the molecular weights of fulvic and humic acids, raising the question of whether the Da unit is perhaps unsuitable for use in molecular weights. Although it is scientifically correct to use dalton for molecular masses, it is more common to use the dimensionless weight units in issues of molecular weights. In soil physics, weight is considered a force and many scientists object to using weight units for expressing the amount of mass. However, according to the second law of Newton, molecular mass is by definition

$$F = ma \text{ or } m = F/a$$

where F = force acting upon a body of matter, m = mass contained in the body, and a = acceleration. On the surface of the Earth, it is commonly accepted that $a = 1$, hence $m = F$. Therefore, force (= weight) = mass, making it perfectly sound on Earth to use weights for molecular masses. The choice depends on the preference of the reader and on the purpose of study; however, nonetheless, it is more practical to express molecular weights in weight units, and preferably in the form of dimensionless units as stated at the start of this section. The dimensionless units for molecular weights, as stated above, is then more user-friendly since they are more easily understandable for a large number of people than Da, amu or " $\times 1.66 \times 10^{-24}$." Nonetheless, the dimensionless figures indeed also show wide variations of molecular weight values for the humic substances.

3.6.2 Issue of HPSEC

Another more important problem is the data used by Piccolo as the prime basis for his introduction of the supramolecular concept in humic acid science. The results of his low-pressure (LPSEC) and especially high-pressure size exclusion chromatography (HPSEC) are claimed to provide indications of humic substances being molecular associations—called by the impressive

name of *supramolecular assemblies*—rather than being polymeric substances. The methods above fall in the category of *gel chromatography*, which is considered a relatively effective method for the selective separation of humic substances according to molecular sizes. However, the molecular size fractionation depends on the type of gel substances used, and a large variety of gels have been applied in the analyses. For the types of gels and principles of gel chromatography of humic substances, reference is made to Tan (2011). The most widely used gel materials are the cross-linked polymers of polysaccharides, polystyrene, polyamides, or the like, distributed under the names Sephadex, Biogel, Cellogel, and the like. Sephadex has been mentioned by Piccolo et al. (2003) as the gel material in their LHPSEC and HPSEC analyses. This kind of gel comes in many different types of size exclusion limits, e.g., to name only a few, in the form of Sephadex G-50, Sephadex G-30, Sephadex G-20, Sephadex G-10, and Sephadex G-2, with a size exclusion limit—in terms of molecular weight—of 50,000, 30,000, 20,000, 10,000, and 2000, respectively. Therefore, the use of the different types of Sephadex will determine the size of humic fractions produced in the analysis. By applying the proper series of Sephadex, any type of molecular sizes can be obtained at will from high to low molecular weights. Because the various humic fractions with different sizes are the results of the analytical operations using different types of gels, the various humic assembly fractions are then only operational assemblies; hence, we are back to the old operational concept. The difference is only that the new concept considers the humic fractions to be molecular associations or molecular assemblies, whereas the old concept calls them humic acid polymers. The humic substance itself is a natural substance, but it is the several molecular fractions, separated by size exclusion chromatography with Sephadex, that are the operational or artificial fractions. The method does not solve the issue of finding a characteristic molecular weight value of humic substances, as also admitted by Piccolo et al. (2003). It tends, however, to support the presence of humic assemblies with molecular sizes varying in a continuum.

The above method then forms the basis of HPSEC used by Piccolo and coworkers for deriving their theory on humic acid assemblies. In gel chromatography, large molecules are eluted first and smaller molecules are eluted last, as is also the case in HPSEC. Piccolo and coworkers contend that when the pH of their humic solution is decreased by adding monocarboxylic, dicarboxylic, or tricarboxylic acids, the amount of large molecules eluted first is reduced, whereas that of the smaller molecules eluted last has increased substantially. This is taken as an indication that the large molecules were

disassembled into smaller molecules by the low pH, conforming to the principles of the supramolecular assembly concept. However, the apparent lack of additional follow-up investigations—necessary for confirming their conclusions—has left the results of their analyses open to attack by disturbing questions. For instance, the possibility that the smaller fragments are broken down by hydrolysis, due to the pretreatments of their humic acid samples used in their HPSEC analyses, has neither been considered nor examined at all. In addition, the chances for the broken segments to form “new” perhaps smaller humic acid assemblies in the fraction eluted last are not explored, and their presence or absence is not established or confirmed by separate analyses. The issues above are very critical since self-assembling, disassembling, and self-reassembling processes are the cornerstones of the supramolecular concept.

3.7 Nanotube Membrane Concept

The nanotube membrane concept was introduced in 2011 by Tan as a result of his discovery of nanotube and nanotube membrane structures of humic acids in his scanning electron microscopic investigations (Tan, 1985, 2011a, b).

3.7.1 Nanochemistry

Nanochemistry is the newest discipline of chemistry in nanoscience, examining assemblies and reactions of atoms and molecules at the scale of 1 nm (1×10^{-9} m) to 1000 nm. A value of 100 nm has also often been stated as the upper limit, although this does not appear to be an absolute cutoff point (Cademartiri and Ozin, 2009; Prasad, 2008). In other words, it is chemistry at the nanoscale and is generally considered evolved as a normal scientific advancement in modern times. However, some people believe that the basics were already applied as far back as in the ninth century on the banks of the Tigris and Euphrates rivers in Asia Minor for producing the shiny luster of pottery. However, many historians attribute the rise of the distinctive Muslim-style pottery in Iraq (Mesopotamia), Syria, and Persia to Chinese influence. Chinese pottery and ceramics were known since the Palaeolithic era, with glaze reportedly produced for the first time in the green glaze ceramics of the Han dynasty in circa 202 BC–220 AD (Lane, 1947; Bushell, 1977). This so-called glaze is caused by a film of metallic nanoparticles applied in the form of salts by the ancient craftsmen on the surface of their pottery. The physical properties of materials change as their sizes approach

the nanoscale. For example, the “nanocolors” of a normally yellow-gold and that of gray silica are red. The green glaze of Han ceramics—to imitate the green shade of jade favored by Chinese emperors—is produced by adding iron oxide in the glaze mixture. Although such a change in physical property is well known in physics, it has remained unnoticed or has not attracted attention until late in the twentieth century. Starting in the 1970s–1980s, a number of events occurred almost at the same time related with the emergence of the new ideas, making it difficult to state what is published first and which follows later as indicated below. Nanochemistry is in the category of *nanotechnology* (or *nanotech*), a term first introduced in 1974 by Norio Taniguchi, but became popular after it was used and promoted by Drexler’s book in 1986 (Drexel, 1986, 1991). The nanoconcept has attracted great attention since then and seems to increase in importance with the development of the scanning tunneling microscope in 1981 by Gerd Binnig and Heinrich Roher at the IBM Research Laboratory in Zurich, Switzerland, enabling analyses of material surfaces at the atomic levels. The two scientists received in 1986 the Nobel Prize in Physics for their invention. The discovery of fullerene, a nanotube particle, in 1985 by Harry Kroto, Richard Smalley, and Robert Curl—who were awarded in 1996 the Nobel Prize in Chemistry—has perhaps not only contributed to the growth of nanochemistry, but has also added more controversies to its applicabilities as a new science in chemistry. At the early stages of growth, the significance and implications of nanoscience were surrounded by many uncertainties as reflected by the debate between Drexel who has promoted, and Smalley who was skeptical about the new ideas (see Schulze, 2003). Nevertheless, this new science has attracted since then considerable research attention from industry because of its important applications in medical, optical, electronic, and other fields. Evidence of the potential benefits of the application of nanochemistry has been reflected, e.g., in the production of antibacterial agents, transparent sunscreens, and stain-resistant textiles. Nanotechnology is now expected capable of creating many new materials based on molecular self-assembly from developing new materials at the nanoscale to direct control of compounds on the atomic scale.

3.7.2 Nanoparticles

Nanoparticles are defined in nanochemistry as particles with diameters between 1 and 100 nm. Larger particles, with size ranges between 100 and

2500 nm, are classified as *fine particles*, whereas those in a size range of 2500 to 10,000 nm are called *coarse particles* (Kiss et al., 1999; Buzea et al., 2007). The very fine particles, defined above as nanoparticles, were originally known as *ultrafine particles*; however, with the development of the science of nanotechnology, the name nanoparticle was introduced and subsequently adopted as the preferred name in the 1990s. Nanoparticles are considered to have the capacity to self-assemble spontaneously into assemblies varying widely in size and length. The size of supramolecular assemblies formed is reported to range from nanometers to micrometers ($1 \mu\text{m} = 1 \times 10^{-6} \text{ m}$). Molecular self-assembly is a concept of *nanofabrication* creating supramolecular associations with a distinct form of structure. Therefore, contrary to Piccolo's idea of randomly produced assemblies (Piccolo, 2002), nanofabrications yield assemblies with well-ordered structures and identifiable shapes and form. At the smallest end of the size scale, they may often appear as clusters, whereas at the other (coarser) end of the scale they can take the shape of tubes, spheres, rods, cups, and/or fibers, to name a few. They are, therefore, often identified and named according to their characteristic shape or form. *Nanoclusters* are usually 1–10 nm in dimension, whereas *nano powders* are composed of ultrafine particles. Nanoparticles containing both hydrophilic properties at one end and hydrophobic characteristics at the other end are available. They are not only of importance as surfactants and for stabilizing emulsions, but also of particular interest in the formation of micelles and membranes in humic acid chemistry. Long carbon nanotubes are also present as one of the larger nanoparticles.

3.7.3 Nanotubes and Nanotube Membranes

Nanotubes are defined as nanoparticles possessing tubular structures, but exhibit tube lengths that can be measured in micrometers ($1 \mu\text{m} = 1 \times 10^{-6} \text{ m}$) or longer (Whitesides and Boncheva, 2002). They can occur naturally or are artificially produced and can be distinguished into several types, e.g., carbon nanotubes, membrane nanotubes, nanotube membranes, and DNA nanotubes. Other examples are nanofibers as in assemblies of peptide amphiphiles and dendritic dipeptides exhibiting hollow cylindrical structures in biological materials. Inorganic types of nanotubes have also been discovered in mineral deposits, composed of metal oxides but with a similar cylindrical morphology as carbon nanotubes. *Nanotube membranes* are, by definition, either single nanotubes with open ends or are membranes composed of a series or an array of nanotubes arranged into a film that is oriented perpendicularly to the surface of a film matrix, like the cells of a bee honeycomb. In contrast, *membrane*

nanotubes are single tubes connecting cells to cells in plant and animal tissue, extending sometimes for >100 µm between cells. They are formed from the cell's plasma membrane and believed to facilitate cell-to-cell long-distance communication. They are also noted to function as a vesicular transport system, allowing transfer of nucleic acids and the flow of other components from cell to cell (Davis and Sowinski, 2008). By weathering or decomposition of plant and animal tissue, it is expected that these membrane nanotubes can be broken down and released into small pieces of carbon nanotubes.

Carbon nanotubes are carbon atoms arranged as tubes with diameters at the nanoscale. Although approximately 10,000 times thinner than human hair, these tubes can range from a micrometer (µm) or less to a few millimeters (mm) in length. They are generally considered to be formed by curling or warping of monolayers of graphite, called *graphene*. However, it appears that the process of warping can also yield imperfect tubes or cylinders and the so-called carbon nanotubes can assume many types of shapes and forms, such as, e.g., sheets, warped sheets, ribbons, ellipsoids, hollow spheres, and other related bended nanostructures. Some examples of their characteristic structures are shown in Figure 3.2. The name nanotube implies that the molecules should be tubular or cylindrical; hence, the name

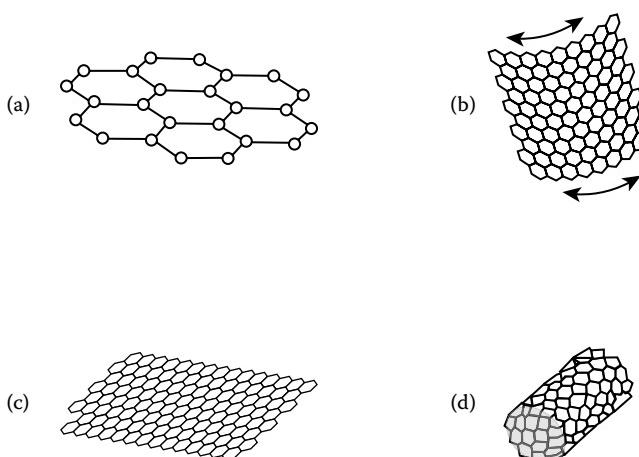


Figure 3.2 Examples of carbon nanotubes. (a) Monolayer of graphite, called *graphene*, origin of carbon nanotubes. Note the hexagonal pattern of benzene structures forming a “benzene” or “phenol” polymer. (b) Warped graphene, called carbon nanotube sheet. (c) Nanotube membrane or bilayer nanotube sheets. (d) Curled or rolled-up graphene, called carbon nanotube. (b, d: From http://en.wikipedia.org/wiki/Carbon_nanotube, accessed July 7, 2011. c: From http://en.wikipedia.org/wiki/nanotube_membrane, accessed October 19, 2012.)

fullerenes is apparently used to cover this group of nanotubes with various shapes and forms. A well-known fullerene discovered in 1980 by Sumio Iijima is the so-called buckyball (Iijima, 1980). Its spherical structure, showing a peculiar pentagonal pattern, has been imitated in the production of footballs of F.I.F.A (Fédérale Internationale de Football Association), referred to as soccer balls in the United States. It is also called *buckminsterfullerene* by Harold Kroto, Richard Smalley, Robert Curt, James Heath, and Sean O'Brien, who created it artificially at Rice University (Kroto et al., 1985).

3.7.4 Humo–Nanotube Associations

The great variety of nanotubes grouped under the name of fullerene as discussed above, including nanotube fibers, nanotube membranes, and many others, occur in nature. These nanotubes can be formed by the decomposition of plant and animal tissue. As pointed out earlier by Wershaw (1999), decomposition and depolymerization of biopolymers in plant and animal tissues will break them down into smaller pieces. The present author believes that such a decomposition process will no doubt also yield a lot of nanoparticles capable of self-assembling. The presence of peptides and other amphiphilic nanofibers, as indicated earlier, is essential in the formation of nanotube membranes. Together with carbon nanotubes, they are essential in the production of humic substances by self-assembly. The hexagonal arrangement of carbon—closely resembling benzene or phenol monomers—in the carbon nanotubes provides the aromatic carbon structure, whereas the peptide nanofiber and membranes satisfy the requirement for the synthesis of humic substances. The concept may look closely related to the conventional ligno–protein polymer theory. However, a stark difference is that the latter underscores polymerization involving strong intermolecular chemical bonding, whereas the humo–nanotube concept considers molecular self-assembly with weak chemical bonds as the main forces.

Evidence for the presence of humo–nanotube associations in natural ecosystems has been presented recently by Tan (1985, 2011a, b). From his recent studies with fulvic acid, isolated from a Cecil soil (Hapludults) in Georgia, United States, the author notices it to possess a network structure of nanotubes. Analyses by scanning electron microscopy reveal structures resembling those of fishnets or the pattern of chicken wire, characteristic for nanotubes (Figure 3.3). Additional evidence is obtained from analyses of aquatic fulvic acid isolated by the author from humic water of the Satilla River in South Georgia, United States. The long cylindrical fiber-like aquatic

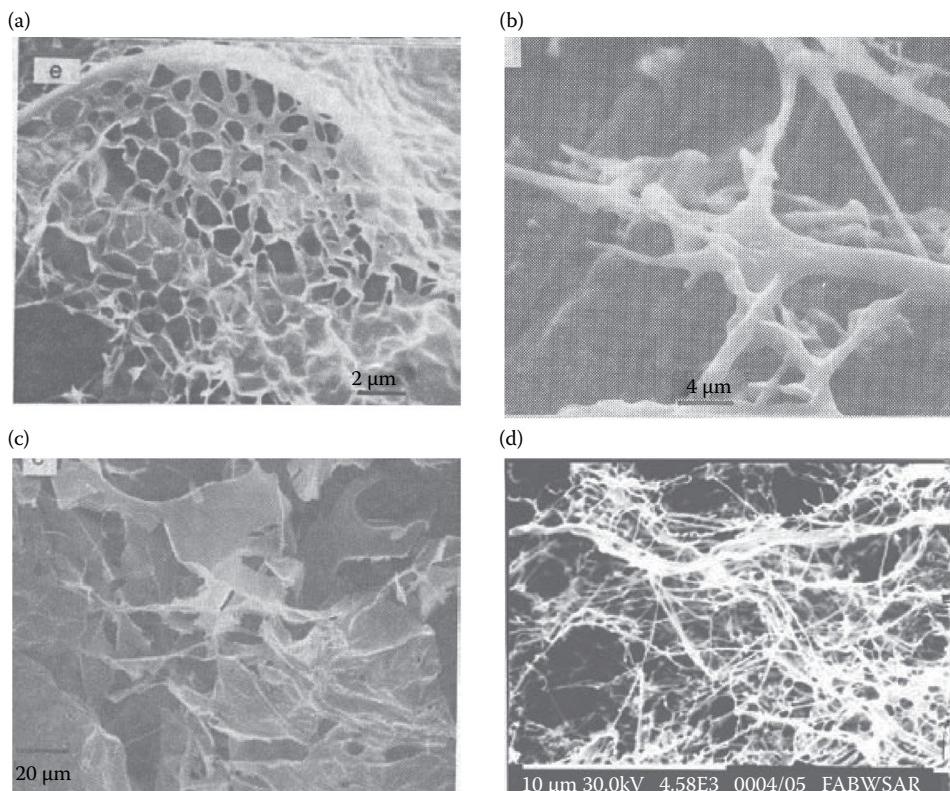


Figure 3.3 Scanning electron micrographs of fulvic acids from a Cecil soil (Hapludults) showing (a) a “fishnet network” or “chicken-wire” pattern, closely resembling carbon nanotube structures presented in Figure 3.2; (b) carbon nanotube cylindrical fibers; and (c) nanotube membrane film. (d) Scanning electron micrograph of aquatic fulvic acid from humic water of the Satilla River, South Georgia, United States, showing carbon nanotube fibers, attracted together into nanotube bundles. (Micrographs from present author’s files; see also Tan, K. H. The new look and nanotube concept of humic acids. Soil Science website—Dr. Kim H. Tan: <http://drkhtan.weebly.com>, Copyright by author, U.S. Library of Congress, Washington, DC, 2011; Tan, K. H. Humic acid nanotube membranes as revealed by scanning electron microscopy. Soil Science website Dr. Kim H. Tan: <http://drkhtan.weebly.com>, Copyright by author, U.S. Library of Congress, Washington, DC, 2011.)

fulvic acid molecules, called nanofibers, are clearly shown and are attracted together by van der Waals forces assuming nanotube bundles (Figure 3.3d). The latter resembles closely a scanning electron micrograph of carbon nanotube bundles produced in the laboratory (Tan, 2011a). On the other hand, humic acid, extracted from lignite, shows the presence of nanotube membrane structures as manifested in scanning electron microscopy by the

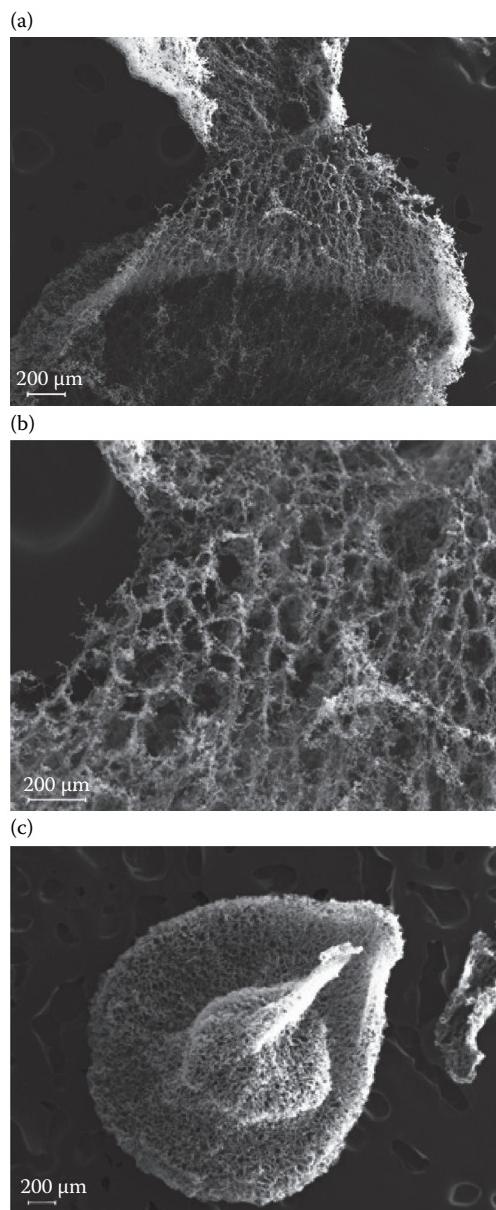


Figure 3.4 Scanning electron micrographs of humic acid isolated from lignite exhibiting a (a) nanotube membrane structure with a torn or ruptured cylindrical assembly, exposing the characteristic honeycomb pattern of a beehive; (b) close-up of a membrane section above showing the honeycomb assembly at higher magnification; (c) humic acid nanotube membrane with a spherical or ball-like structural arrangement, resembling a nanobud. (Micrographs from author's personal files; see also Tan, K. H. Humic acid nanotube membranes as revealed by scanning electron microscopy. Soil Science website Dr. Kim H. Tan: <http://drkhtan.weebly.com>, Copyright by author, U.S. Library of Congress, Washington, DC, 2011.)

distinctive honeycomb pattern of a beehive (Figure 3.4a). Nanobuds are also detected occasionally, suggesting involvement of buckminsterfullerenes or buckyballs in structure formation of humic acids, although buckyballs are characterized mostly by a pentagonal structural pattern, as indicated earlier. In supramolecular chemistry and nanoscience, nanobuds are fullerenes assembled and bonded by covalent bonds on the outer walls of nanotubes as shown in Figure 3.4c. Nanobuds are supposed to function in anchoring the nanotubes and, hence, in the author's opinion, in stabilizing the molecular structure of the nanohumic assembly.

This humic acid nanotube membrane structure is presumably the most stable thermodynamic association that can be attained by self-assembly. Such a more orderly structural assembly relates better than Piccolo's random bundles for explaining issues of consistent carbon and nitrogen contents and reproducible spectroscopic features of humic substances.

Chapter 4

Nature and Distribution of Humic Matter

4.1 Historical Background: Era of Ulmic, Crenic, and Geic Acids

Humic matter is composed of a variety of substances that can be obtained by extraction and fractionation on the basis of their solubility in alkaline and acidic solutions. A number of scientists cited Franz Karl Achard for extracting in 1786 the first humic substances from peat and bogs in Germany, followed in 1797 by Louis Nicolas Vauquelin, who allegedly isolated them from plant materials (Susic, 2008; Stevenson, 1994; Russell and Russell, 1950). However, it was rather the work on humus and its extraction in 1826 by Carl Philipp Sprengel in Germany and in particular that of Jöns Jacob Berzelius in 1839 in Sweden that are considered to have provided more relevant contributions for advancing humic acid science. During the early periods mentioned above, an array of names was created to identify the extracted materials, e.g., *ulmin*, *ulmic acid*, *geine*, *geic acid*, and *crenic* and *apocrenic acids*, names that were confusing even for the early creators themselves as well as for other scientists in the following years. Today, a lot of controversies still exist in fact regarding the meaning of Achard's *torf*, Vauquelin's *ulmin*, Sprengel's *humus*, and of the material called *geine* by Berzelius (Achard, 1786; Vauquelin, 1797; Sprengel, 1826; Berzelius, 1839). A number of factors have contributed in creating the controversies. They are in part presumably caused by the purpose of research

in the old days, which is aggravated by issues of today's translations of the books and/or reports from the old German and French languages into English. For instance, in the old German literature, the name *torf* and a term referring to *vegetable mold* or *leaf mold* are often mentioned as the materials used for extraction of humic substances. *Torf* is then often translated into English as *turf*. To most people at present, including the present author, turf assumes the meaning of the "upper stratum of soil held together by grass roots into a thick mat." This is also the definition in the *Webster Collegiate Dictionary*. The American NFL (National Football League) stadiums are often using artificial turf for their indoor football fields, whereas courses in turfgrass are often offered today at many American universities. However, in the context of the German literature, *torf* is just plain *peat*, whereas *turf* is in fact a Dutch name for peat. The old German term for vegetable or leaf mold does not translate also into a fungus producing fibrous-like filaments covering decomposing plant residue—the conventional meaning of mold—but the German authors were referring to *muck* or "unripe" peat. Vauquelin also used *cell sap* for extracting humic-like substances, providing support for the idea that humic compounds—called earlier *proto-humic substances* (see Section 3.1)—can be formed in the plant body. Such confusion in translation and the use of unconventional sources for extraction may give different meanings and/or interpretations in extraction issues of humic materials.

Most of the pioneers in humic science named above are also chemists, physicians (medical doctors), or pharmacologists. Both Achard and Vauquelin were not researching humic acids but were looking for chemicals. Vauquelin, for example, was a pharmacologist and was looking for therapeutic pharmaceuticals for treatment of ulcers and cancers as can be noticed from the title of his published report (Vauquelin, 1797). Using the brown cell sap, called scientifically *exudates*, of elm trees, he succeeded apparently in isolating a black substance that he called *ulmin* (from *Ulmus* sp. the Latin name for elm trees), providing fuel for the present-day idea of formation of humic compounds within live plant bodies, as indicated above. In solid condition, this substance, called *ulmin*, is insoluble in water and assumes physical characteristics reflecting a shiny metallic black luster, similar to humic-like compounds obtained earlier by Achard from the alkali extraction of peat (Achard, 1786). Sprengel (1826), on the other hand, was at first indeed studying humus and its influence on plant nutrition and growth. He is known for the distinction of humus into acid and mild humus as

discussed earlier in Section 2.3.2.1. However, at a later stage, he apparently abandoned his humus theory and developed the inorganic or mineral theory in plant nutrition. Sprengel was credited, in fact, for being the first to formulate the *law of the minimum* in plant nutrition. Liebig has allegedly only popularized this fundamental law, and proposals are recently floated around to rename the law as the *Sprengel–Liebig law of the minimum* (Van der Ploeg et al., 1999).

In 1839, Berzelius isolated organic substances from spring water in Sweden, which he named *crenic acid* and *apocrenic acid*, names derived from the Greek term *kpnvn* (= spring or fountain). These acids were allegedly the decomposition products of plant materials present in spring water. A similar extraction of *mold* also yielded the same yellow acidic mass, called *geic acid* (from Greek *geine* = ground, earth) by Berzelius (1839). The substance called ulmin by Vauquelin was renamed geine or gein by Berzelius because of its close similarities with his substance isolated from mold (muck). The term geine is used in the sense of the present term humus. Many consider Berzelius also as one of the founders of chemistry at the level of Antoine Lavoisier and John Dalton for his contribution in developing and using chemical symbols, the same basic concept chemistry is using today. However, his chemical notation using superscripts, e.g., H^2O , has been changed into the present-day notation applying subscripts, e.g., H_2O (Berzelius, 1839). Although Justus von Liebig disagreed with Berzelius' work, and in particular on the effect of humus and its humic components in plant nutrition, the investigations of Berzelius were continued in the Netherlands by Gerardus Johannes Mulder (1840), professor of chemistry at the University of Utrecht, the Netherlands, and author of the book *De Scheikunde der Bouwbare aarde*, which has been translated into English (see Mulder, 1862). Apparently, Mulder was associated earlier with Berzelius in the investigation of protein. He succeeded in the following years in isolating additional humic fractions by extraction of peat, *torf* (turf), and decomposed plant materials in the Netherlands. On the basis of color and solubility, Mulder (1862) classified them into the following:

1. Ulmic acid and humic acid, the brown and black fraction, respectively, soluble in alkali but insoluble in acid.
2. Crenic acid and apocrenic acid, the yellowish to brown fractions soluble in water as indicated above for geic acid. They are the fulvic acids of today.

3. Ulmin and humin, the fractions soluble after heating in alkali but insoluble in cold alkali, acid, and water. Ulmin is also related to gein or geine and believed capable of yielding ulmic acid, hence the latter name indicating its origin from ulmin.

Mulder believed that in the decomposition of soil organic matter, ulmic acid is formed first, which he assigned a molecular formula of $C_{20}H_{14}O_6$. Oxidation in soils results in successive transformation of ulmic acid into humic acid, geic acid, apocrenic acid, and the latter finally into crenic acid. These compounds are given the chemical formulas—using today's modern notations—of $C_{20}H_{12}O_6$, $C_{20}H_{12}O_7$, $C_{24}H_{12}O_{12}$, and $C_{12}H_{12}O_8$, respectively. Mulder's accomplishment was followed 27 years later by the discovery of *hymatomelanic acid*, isolated by Hoppe-Syeler (1889) as the ethanol-soluble fraction of humic acid. After that, no further achievements of major importance can be noted in the study and isolation of humic substances until Sven Oden's (1914, 1919, 1922) concept surfaces at the start of the twentieth century. Oden's concept, considered by many scientists as based on a more solid scientific foundation, was in essence a revision of Mulder's ideas and classification. His book (Oden, 1922) provides a definition of humus that is supposed to be a newer version deviating in concept from that introduced one and a half centuries earlier as an important issue in plant nutrition by Wallerius in 1761 (Russell and Russell, 1950). However, some cited de Saussure in 1804 for the introduction of the term "humus" (Frimmel, 1991). Because today's theories of humic acid science are—according to many scientists—applying some of the basics of Oden's ideas, some of the major definitions are given in abbreviated forms below for the purpose of completeness and comparisons. They are translated from the old German language used in his book into English by the present author. Humus is defined by Oden as the organic matter in soils composed of fresh and decomposed plant materials, and such a definition is, in the author's opinion, comparable to what we call now *litter* (see Chapter 1, Sections 1.1.2 and 1.3.1.3, and Chapter 2, Section 2.1). *Humus stoffe* (= literally humus "stuff" or humus matter) is, in Oden's words, the new "stuff" formed by the action of air and water (meaning by oxidation) on humus, producing new dark-colored materials, fertile and beneficial for agricultural soils. This is then more compatible with our modern-day concept of humus (see Chapter 1, Section 1.1.2). *Humus acids* (*humussäuren*) are formed by humification of plant residues. Oden noted that especially pectin and pectic acids will yield humus acids upon humification. The term *humus acids* is apparently used to cover the mixture of acidic substances present in humus,

such as *huminsäure*, *hymatomelan säure*, *ulminsäuren*, and *geic säuren* (see also Chapter 2, Section 2.3.1).

4.1.1 Nature of Humic Substances in the Early Twentieth Century

In his revisions of Mulder's older theories, Oden tried apparently to bring some order to the confusing nomenclature by reclassifying the array of humus acids with new names as follows:

1. Fulvic acid, which replaces the use of the terms crenic acid and apocrenic acid. The name is allegedly derived from Latin *fulvus* (= golden yellow).
2. Humic acid (*huminsäure*) for the fraction soluble in alkali and insoluble in acid.
3. Hymatomelanic acid, a name used earlier by Hoppe-Syeler (1889) for the humic acid fraction soluble in ethanol. However, since alcohol is not present in large amounts in nature, Oden also thinks it is better to include *hymatomelan säure* into *huminsäure* and call the mixture *humus säure* or humus acid.
4. Humus coal, for replacing humin and ulmin.

Oden's theory, although challenged many times over the years, has set the stage for the development of the concepts and types of humic substances followed today. Except for humus coal, his concept has weathered the criticisms of time, and the names fulvic acid, humic acid, and hymatomelanic acid are still in use today. Oden's idea on humus and humic substances triggered in the beginning a flurry of investigations, and the ligno–protein theory was introduced during this period by a number of researchers (Fuchs, 1930a, b, 1931; Hobson and Page, 1932a, b, c; Waksman, 1938), a concept that has dominated humic acid chemistry and formation until today. This theory assumes humic matter to be the product of reactions mainly between lignin and protein, two important components of plant tissue. Although it is a very viable theory that is used by Flaig (1975) as the foundation of his humic acid concept, other theories were presented when more became known about humic acid chemistry toward the end of the century. This will be discussed in more detail in Chapter 5 on biochemistry and formation of humic matter.

The new burst of research activity has also resulted in the development of new names, often amounting only to noise in the nomenclature of humic

substances. Names such as *rotteprodukte* (rotten or decomposed products) and *echte huminsäuren* (real humic acid) were proposed by Simon and Speichermann (1938). Springer (1938) added *humoligninsäuren* and *lignohuminsäuren* (lignin- or lignohumic acid) to the confusion. Attempts, especially of fractioning humic acid further into several subtypes, have come up with more names. By manipulation of the fractionation procedures, it has been reported that humic acid can be subdivided into α - and β -*humic acid* (Russell and Russell, 1950). The name α -humic acid is used for the initial or original humic acid fraction before ethanol extraction for isolation of hymatomelanic acid, whereas the humic acid residue, remaining after ethanol extraction, is called simply humic acid. The name β -humic acid was reserved by Waksman (1936) for a precipitate produced after adjusting the pH of a fulvic acid solution to 4.8 with NaOH. According to Stevenson (1994), this β -fraction is an Al-humate with properties similar to an organic substance obtained by Hobson and Page (1932a, b, c) named *neutralization fraction*. Not only is such a nomenclature confusing, but also the identification of a fulvic acid fraction as a humate is questionable. It is also difficult to call it an *Al-fulvate*, since it is insoluble in acid condition. Hence the only alternative is the possibility that the separation of humic acid from fulvic acid in the soil extract has been conducted improperly or a proper fractionation procedure is indeed unavailable. Consequently, the analysis is fraught with many errors or uncertainties, justifying claims for the production of artifacts. Although several people agree with α -humic acid as defined above, it makes more sense if this name is assigned to the insoluble part of humic acid remaining after ethanol extraction. This residue has changed in composition and it seems more reasonable to assign it the symbol α and retain the name humic acid for the original substrate before ethanol treatment. Hence, humic acid can be fractionated by ethanol into a α -fraction and hymatomelanic acid. In analogy to the above, reference can be made to the division of humic acid into a brown and a gray fraction, which has attracted considerable attention in Germany. By using neutral salt solutions, Springer (1938) succeeded in separating humic acid into (1) a brown fraction (*Braunhuminsäure*), soluble in NaCl, and (2) a gray fraction (*Grauhuminsäure*), insoluble in NaCl. The brown humic acid is said to be highly dispersible, contains a lower carbon content, and according to Stevenson (1994) has characteristics of humic matter in peat and in brown coal. However, Kononova (1966) is highly critical of humic matter originating from coal or peat, since these two materials are formed in anaerobic conditions completely opposite to the aerobic system present in soils

responsible for the formation of soil humic matter. Springer's gray humic acid has a low degree of dispersion and is easily coagulated. In Stevenson's opinion, it looks similar to the humic acid in mollisols.

4.1.2 New Developments at the Turn of the Century on the Nature of Humic Substances

With research interest declining among soil scientists who faced resentment owing to an apparent identity crisis on humic acids late in the twentieth century, as discussed in Chapter 3, scientists from a wide variety of other disciplines have taken the lead in humic acid research. Chemists, geochemists, hydrologists, environmentalists, ecologists, and even geologists have become fascinated by the ubiquitous presence of humic matter in the ecosphere. In contrast to most soil scientists, they recognize its profound role in environmental issues, and its effect on migration and immobilization of industrial and nuclear waste and other pollutants. In industry, medicine, and pharmacy, humic substances are recognized as potential sources for the production of valuable chemicals. They are considered commercially viable for use as surfactants and as drilling fluids in oil exploration, as well as in medicines for human health. Research in humic matter took a sharp turn away from soil science to make rivers, lakes, and oceans the centers for explorations and investigations of humic substances. The result is that new humic compounds, assigned exotic names, have been discovered, enlarging our concept of humic matter. The name *copropel* is presented for a humic substance, labeled as humus by Swain (1963) and Stevenson (1994). It has been formed from the decomposition of microscopic plants in eutrophic lakes and marshes. A black mass of humified material located at deeper hypolimnetic areas of lakes and bays is called *sapropel*, whereas a pondweed type of sapropel, believed to originate from cellulose-rich plants, is called *förrna* by the above authors. A deposit in dystrophic lakes consisting of an allochthonous precipitate of humic acid and detritus is referred to as *dy*. Marine slime resulting from settled decomposed plankton detritus is called *pelogoea* and an amorphous, gummy accumulation of humic substances beneath or within peat bogs is *dopplerite*. Recently, less exotic names have been used for humic matter present in the water medium. It is known by geochemists under the collective name of *aquatic humic materials*, as opposed to *terrestrial humic materials* for humic compounds in soils (Christman and Gjessing, 1983).

A new science of aquatic humic acid seems to have emerged somewhat rapidly and in particular through the great efforts of Leenheer, Thurman,

and Wershaw, geochemists of the U.S. Geological Survey (USGS), Denver, Colorado, United States, as discussed in Chapter 1, Sections 1.3 and 1.4 (Thurman and Malcolm, 1981; Leenheer and Croué, 2003; Wershaw et al., 2005). Humic substances in aquatic ecosystems have, in the beginning, not attracted too much attention and remained unknown for a long time, perhaps because of their alleged low concentrations (Frimmel, 1991). However, aquatic humic substances were, in fact, isolated for the first time way back in 1839 by Berzelius from spring water and given the names crenic and apocrenic acids, as discussed above. Their presence in ecosystems is now noted to be very obvious by the yellowish colors of natural waters, identified variously by different names, such as fulvic acids by Oden (1922), yellow organic acid by Shapiro (1957), *gelbstoff* (yellow stuff) by Kalle (1966), and aquatic humus by Gjessing (1976). Even at the current stage of knowledge, the definitions and concept of aquatic humic substances are still vague and need more refinement, since the idea of aquatic humic matter seems often not to be different from the concept of DOM (dissolved organic matter). As examined earlier in Chapter 1, the terms aquatic humic acid and DOM are often invariably used interchangeably by limnologists, hydrologists, and geochemists of the USGS in their efforts to promote the importance of DOM. As discussed in Chapter 1, the name DOM implies the existence of organic matter in dissolved condition and may or may not contain humified substances. Such organic matter is present in natural waters in many forms and sizes from small monomers to large polymers that may become aggregated, forming colloids and larger particles. This mixture is usually extracted from natural waters by filtration using a 0.45- μm filter (see Chapter 1, Section 1.3.3). All organic matter passing through is called DOM, whereas that part retained by the filter is called POM (particulate organic matter). The term DOC (dissolved organic carbon) and NOM (natural organic matter) have also often been used to refer synonymously to DOM, increasing the confusion in terminology and concept of aquatic humic substances. A possible scientific or analytical explanation has been provided to reconcile the differences in Chapter 1, Section 1.3. Another possibility is the indications that the use of DOC or NOM for DOM can perhaps also be only a matter of preference between the various authors, but definite evidence for this issue is at present unavailable. Unfortunately, the significance of the XAD method developed earlier by Thurman and Malcolm (1981) for extraction of aquatic humic substances seem to be overshadowed by the allegedly more modern 0.45- μm filtration techniques (Leenheer and Croué, 2003; see also Chapter 1, Section 1.4.1 of this book). Nevertheless, some believe that DOM or DOC

is more likely a mixture composed of a variety of dissolved organic matter, including aquatic humic substances. In natural waters, it is noted that aquatic humic substances typically make up between 30% and 80% of the DOC or DOM (McKnight et al., 1985; Peuravuori et al., 2002). Regrettably, a recently published book by Steinberg (2003) has not contributed toward solving the controversies adequately. Regardless of the impressive title, *Ecology of Humic Substances in Fresh Waters*, Steinberg's idea of aquatic humic substances is based on the premises of theories of terrestrial humic substances that have been formed in aerobic ecosystems (see Steinberg, 2003, Box 1.2, pages 27–30). Chapter 2, on his origin of humic substances in freshwater, also underlines biogeochemical pathways of the biopolymer ligno–protein concept, which brings only more confusion to the aquatic concept rather than solving the controversies. The ligno–protein concept—as discussed earlier to be promoted by Flraig (1975, 1988) and others—is valid only for humic substances in terrestrial aerobic ecosystems. It is now generally realized (see Chapter 1, Section 1.3.3) that the sources for the formation of true autochthonous aquatic humic matter are indigenous aquatic plants, such as algae and phytoplankton, which do not need lignin for growth and are mostly composed of carbohydrates and protein.

The few soil scientists who have continued with humic acid research in soil ecosystems have also yielded some results. An alleged new humic acid fraction, identified by the name *green humic acid*, was isolated by Kumada and Sato (1962). Chromatography using a cellulose column separated a humic acid extract of a spodosol into green and brown fractions. The green fraction, called green humic acid, is believed to be derived from a fungal metabolite (Kumada and Hurst, 1967) and has attracted considerable attention, especially in Japan and New Zealand. In Japan, much value has also been placed in the use of visible light spectrophotometry in the identification of humic substances. The nature of absorption spectra and values of $\Delta \log K$ ($= K_{400} - K_{600}$ in which K = extinction) are applied to distinguish humic acids into four major types, i.e., types A, B, R_p, and P (Kumada, 1965, 1987; Kumada and Miyara, 1973; Yoshida et al., 1978). The P-type of humic acid produces, after separation by gel filtration and column chromatography with cellulose powder or Sephadex, a P_b (brown) and a P_g (green) fraction. The P_g humic fraction corresponds to the green humic acid discussed above. However, the existence of green humic acid is later rescinded by Kumada (1987), who considers the name as incorrect since humic acid is, by definition, brown to black in color. In his opinion, the green fraction is an impurity commonly coextracted with the brown (P_b) fraction.

In the United States, with many of the prominent authorities adhering to the concept of humic substances being operational compounds, no new discoveries have been reported in recent years. Although not really a major breakthrough, Stevenson's (1994) suggestion for distinguishing generic humic substances should perhaps be mentioned in all fairness, although this term had been recognized earlier by Kononova (1966) and Kumada (1987). The name *generic fulvic acid* is used by Stevenson for fulvic acid purified by the XAD resin procedure. Accordingly, he believes that fulvic acid can be distinguished into (1) a generic or true fulvic acid, obtained by purification with Amberlite XAD resins, and (2) a fulvic acid obtained after purification using conventional ion exchangers and dialysis procedures. However, the analogy presented by Stevenson in reference to the subdivision of humic acid by MacCarthy et al. (1979) makes the generic concept a very confusing issue. Peat humic acid is in fact separated into two subfractions by MacCarthy and coworkers using a pH gradient elution technique, whereas Stevenson's generic fulvic acid is just a XAD resin purified version of fulvic acid, involving no separation into subfractions at all. Consequently, the correctness of using the term "generic" is still open to question, and the subdivision of humic acid as discussed above is also suspect. More convincing research data are needed differentiating unequivocally the generic from the conventional type of fulvic acid. No supporting data have been presented confirming Stevenson's contention that generic fulvic acid is lower in carbohydrate and peptide contents than conventional fulvic acid.

In summary, it can be stated that several new concepts on humic matter and a number of new humic substances have been presented or discovered toward the modern era. This has no doubt broadened our perceptions on humic matter science and opens new horizons by increasing our knowledge on the existence of new varieties of humic matter. Some of the new types of humic compounds have been acknowledged and used occasionally today, although several tend to be considered only of academic importance. However, Waksman's (1938) proposal to delete all the names and replace them by humus has not found wide acceptance. On the other hand, Oden's concept on humic acid, fulvic acid, and hymatomelanic acid, and Mulder's idea of humin seem to have weathered all criticisms. The trend can be noticed that they are widely used today by the majority of scientists, although reluctantly by some. Names such as green humic acid, gray humic acid, and brown humic acid have been used sometimes, depending on research priority and interest. Recently, several new theories have been introduced in line with the advances in science and technology of modern

times, e.g., the micellar, supramolecular, and humo-nanotube membrane concepts. They provide new models and undoubtedly bring profound changes in perception and ideas about the nature of humic substances conforming to new standards set in the new century. Older established theories of polymerization in the formation of humic substances are suggested to be replaced by the micellar, the supramolecular chemistry, and more recently by the humo-nanotube membrane self-assembly theory. The new concepts and consequent changes have been examined and discussed in detail in Chapter 3.

4.2 Distribution of Humic Matter

The distribution of humic matter is not limited to the soil ecosystem and climatic conditions. Thought at first to be present only in soils, humic matter is currently assumed to be the most widely distributed organic carbon-containing material on the earth's surface. It is present in soils, streams, lakes, and the oceans, and in their foam and sediments, from the tropics to the arctic regions. Its presence as a major constituent of the huge deposits of peat, lignite or leonardite, coal, and oil shale adds to the dimension of its wide occurrence worldwide. Geochemists are even of the opinion that the greatest storehouses of humic matter are the oil shales (Swain, 1975). Although most of the humic matter is a natural product synthesized in the environment, some are now assumed to be *anthropogenic* in origin, such as in polluted waterways, drainage ditches, and sewage ponds or sewage lagoons. Especially in the "Old World," such as in Europe, anthropogenic humic matter, identified as humus of harbor and city agglomeration sediments, has started to become recognized (Ciéslewicz et al., 1996). No doubt, such types of humic matter also abound in other parts of the world where stable civilizations have had the opportunity to accumulate a lot of organic wastes throughout the centuries.

4.2.1 Humic Matter in Soils

Humic matter in soils is present mostly in the A horizons. The source for its formation is the litter layer lying on the surface of the A horizon. In pedology, this layer of organic matter is called the O (= organic) horizon, which in ideal conditions may be composed, consecutively from top to bottom, of the O (= fresh residue of plant material) and underlain by the Oi (fibric),

Oe (hemic), and Oa (sapric) subhorizons (see Chapter 1, Section 1.3.1). Hence, as indicated earlier in Section 1.3.1, fibric (from Latin *fibra*) refers to the slightly decomposed material, hemi (Greek for *half*) to half-decomposed material, and finally sapric (from Greek *sapros*) to completely decomposed material or humus. The latter is commonly dark gray to black, and this Oa subhorizon forms the contact zone between the litter layer and the mineral soil surface below. Part of the decomposed material in the Oa horizon is transported into the soil's A horizon by the combined actions of leaching and of burrowing animals, insects, and microorganisms, called *detritivores*, where it is thoroughly mixed together with other soil particles (see Chapter 1, Section 1.3.1.3). Earthworms are good examples of detritivores and they are also of special importance in the so-called *cultivation effect* (Tan, 2009). Earthworm casts, containing digested SOM and perhaps also humified material, are deposited on top of the soil's A horizon. Another part of the sapric material in the Oa horizon—the humified fraction—is leached into the soil's A horizon below and also incorporated with the other soil constituents. In most soils, the humic substances together with the coarser humus particles make the A horizon darker in color than the underlying soil's B horizon. Depending on the organic matter content, the A horizon can become gray to black. The exception is the Bh horizon in spodosols; however, this is another story that will be addressed in the following section.

4.2.1.1 Distribution of Humic Matter in Mollisols, Andosols, and Other Soil Orders in the World

Humic matter occurs in all kinds of soils since it is the major fraction of soil humus. Although variations as to its content can be noticed due to differences in climate and drainage, humic matter can be found in all soils worldwide from the lowlands to high in the mountains of warm tropical to frigid arctic climates. It is also present in humid region to arid region soils. Famous for their high organic matter contents are *mollisols*, soils under grass vegetation of the semihumid regions, called the Great Plains in the United States. These soils are called *chernozems* in Russia. Organic matter contents in U.S. mollisols have been reported to be as high as 5% to 6% in terms of organic carbon (Stevenson, 1994). This is equivalent to approximately 9% to 10% organic matter, and half of this is estimated to be humic matter. Most of the humic substances in mollisols are concentrated in the so-called mollic epipedon (*epi* = surface and *pedon* = soil), which is a diagnostic surface horizon with high organic matter content and the blackest in color in the soil profile (Soil

Survey Staff, 1990). The humic fraction in mollisols has a composition characterized by a humic acid content slightly dominating over that of fulvic acid, as noticed by its fulvic acid/humic acid ratio ranging from 0.9 to 0.6 (Tan, 1978).

Another soil with a similar high humic matter content is the *andosol* or *andisol* in U.S. Soil Taxonomy, a soil occurring in the humid tropics to the arctic regions (Arnalds et al., 1995; Tan, 1984, 2008; Theng, 1980). Its humic fraction is concentrated in the so-called andic epipedon, again a surface horizon with andic properties, and the highest organic matter content. It is also the blackest in color among the horizons in the soil profile. However, the composition and type of its humic matter differ markedly from those of the mollisols. The fulvic acid content makes up more than half of the humic matter in andosols, which is in sharp contrast with that in mollisols. The fulvic acid/humic acid ratio is often noticed to range between 4.0 and 1.0 in andosols of the humid tropics (Tan, 1964, 1965, 1984). Another marked difference is that the humic matter in andosols is present in close association with aluminum and allophane, whereas the humic matter in mollisols is more likely present as Ca-humate and Ca-fulvate.

Large amounts of humic matter are also noticed in *spodosols*, where they are concentrated in the B_h or *spodic horizons*. This is in contrast to mollisols and andosols where the A horizons contain most of the humic matter. The B_h horizon deposit of humic matter is often so thick that it becomes a valuable source for commercial humate production, as is the case in Florida (Lobartini et al., 1992; Burdick, 1965). Spodosol humic matter has been a favored material for investigations in Canada, where the results are taken to apply also for humic matter from other soils (Schnitzer and Khan, 1972; Schnitzer, 1976). Judging from its formation due to leaching from the A and E horizons, the general opinion is that spodosol humic matter is composed of large amounts of fulvic acids. However, recent studies with a spodosol, on the border of Georgia and Florida, reveal its humic matter to have a composition characterized by an FA/HA ratio of 0.13, suggesting a humic acid concentration 10 times higher than that of fulvic acid (Lobartini et al., 1991). This finding supports an earlier report for the Unicamp Company in Florida showing that state's source of its humate products to contain 91.3% humic acid and 8.7% fulvic acid (Tan et al., 1988). Since spodosols are very acidic soils, conditions favoring dissolution of large amounts of Fe and Al, the humic substances are mostly in the form of Fe- and Al-humates and fulvates.

Other groups of soils containing humic matter are the *ultisols* and *oxisols*, soils generally low in organic matter content due to a rapid rate of decomposition. The oxisols of the humid tropics are notorious for their low organic

matter contents, with contents in the A horizons often reported to be as low as 1% organic carbon (Tan, 2008). The humic matter in these soils is often noticed to contain more fulvic acid than humic acid, and these substances are assumed to be present as Fe- and Al-fulvates or humates (Tan, 1978, 2008). However, the lowest organic matter contents are detected in *aridisols*, soils of the arid regions, and the sandy soils in deserts. Because of deficiency of water, biomass formation and decomposition reactions in these dry regions are very limited.

4.2.2 Humic Matter in Soils of Wetlands

Wetlands are land areas that are permanently or seasonally saturated with freshwater, brackish water, or seawater. They can be formed artificially or can occur naturally, such as in the coastal mangrove swamps in the Florida Everglades and the marshlands of the Okefenokee swamp in South Georgia, United States. Large areas of wetlands are also reported to occur in the cold arctic regions of the world; in the West Siberian Plain; in the Amazon River basin of Brazil; and in Paraguay, Bolivia, and other regions in South America. They include marshes, swamplands, bogs, and fens. Peat bogs are freshwater wetlands, whereas marshlands usually develop along the banks of rivers and lakes. Because of the flooding, ponding, and saturation with water, the soils are characterized by anaerobic conditions in the surface and other parts of the pedon. They are known often under different names, such as *hydromorphic soils* or *hydric soils*. In U.S. Soil Taxonomy, they are referred to as soils with an aquic soil moisture regime (Soil Survey Staff, 1990). Because of the presence of anaerobic conditions, these soils generally support a distinctive hydrophytic vegetation, scientifically known as *hydropytiae*, which in plain language means aquatic plants, e.g., water lilies, sedges, and cattails. Often, many of the hydrophytes have *aerenchyma*, or internal spaces in stems and rhizomes, for transport of atmospheric oxygen to the roots (Keddy, 2007).

Under favorable conditions for large accumulation of organic matter, organic soils are formed in wetland areas. Under the influence of poor drainage, anaerobic decomposition of plant residue may prevail in soils of the wetlands (Tan, 2000). Because of lack of oxygen, the decomposition process is slowed down, if not inhibited, and incomplete, and hence contributes to accumulation of huge amounts of organic residue. Many of the wetlands and some of the lake areas are also eutrophic, encouraging excessive growth of aquatic weeds and other plants. The latter provides an overabundance of

dead organic residue filling gradually the inundated or wetland areas. The partly decomposed organic matter will eventually develop into bogs, peat, and muck, which contain large amounts of humic matter. They are also often believed to be precursors for the formation of coal and ultimately fossil fuel (Hatcher et al., 1985). When conditions are favorable, sapropel, copropel, and the like may also develop into peat and bogs. The only conditions required are anaerobic environments for the accumulation and subsequent development into peat and its eventual conversion into coal. These soils composed largely of accumulated organic matter are called *organic soils* or *histosols* (Latin *histos* = tissue) by the U.S. soil taxonomic system. In contrast to a mineral soil system, where the organic matter content makes up only a small fraction compared with the mineral fraction, the organic matter content is an integral and substantial part of peats or histosols. By definition, organic soils contain >80% organic matter and <20% mineral matter (Brady and Weil, 1996). A more complex definition, used in soil taxonomy (Soil Survey Staff, 1990), indicates that organic soils must have an organic carbon content >18% in the presence of >60% clay, or >12% in the absence of clay.

Peat deposits are not limited to climatic conditions and can be found worldwide where large amounts of biomass are available and where decomposition of organic residue is inhibited by either excess water or low temperatures, or both. As discussed above, peat refers in general to organic soil composed mostly of organic residues from the very slow decay of generations of aquatic vegetation, such as marsh grasses, mosses, and bog mosses called often sphagnums, and sedges, and including a great variety of shrubs (Johnson, 2008). In the humid tropics of Southeast Asia, trees make up the dominant vegetation of coastal peatland areas (Tan, 2008). Important peat areas in the United States are located only in (1) South Florida, because of the presence of excess water in the surrounding of the Everglades, and (2) near the Great Lakes in the areas of Michigan and surroundings where both excess water and low temperatures are the reasons for slow decay and resulting accumulation of organic residues. However, the two regions of peat soils are not that extensive as the moors and heathlands covered with peat or turf soil in Ireland, Scotland, North Germany, the high plains of Bavaria, Denmark, and the Netherlands. As indicated above, peat is also distributed from the tropical Amazon basin of Brazil and the coastal regions of Sumatra and Kalimantan (Borneo), Indonesia, to the Baltic coast in Europe and tundras in Alaska and other arctic regions. In addition to excess water, the frigid temperature in the arctic tundras is another reason for inhibiting decomposition of organic residue. In his opening address at the 1972 International

Meeting of Humic Substances at Nieuwersluis, the Netherlands, Golterman (1975) underscored the importance of peat as the producer of humic substances affecting the living environment of the Dutch people.

Called sometimes peatlands or mires in Europe and Canada, and known as histosols in the United States, these organic deposits are believed to cover an area of 500 million hectares worldwide, representing an organic carbon reserve of 10^{12} metric tons (Mathur and Farnham, 1985), and only the organic carbon reserve in oil shale is believed to exceed this amount. Swain (1975) presented data showing the earth's crust to possess a total organic carbon reserve of 19×10^{15} metric tons, and most of it (18×10^{15} metric tons) is stored in oil shales.

Most of the information indicates that peats contain large amounts of humic acids (Zelazny and Carlisle, 1974; Kononova, 1966), although occasionally it is reported that humic matter in peat is composed mostly of fulvic acids (Schnitzer, 1967). The humic acid content appears to increase from peat to muck, with the more humified muck noticed to contain its humic matter mostly in the form of humic acid (Preston et al., 1981). The elemental composition, spectral characteristics, and other chemical properties of peat humic acids are believed to be similar to those of humic acids in mineral soils (Mathur and Farnham, 1985). However, as indicated before, Kononova (1966) was highly critical of humic matter originated from peat, since the material has been formed in anaerobic conditions, completely opposite to the aerobic system present in soils.

4.2.3 Humic Matter in Aquatic Environments

It is now an established fact that the distribution of humic matter is not limited to the soil ecosystems only, but it has also been detected in streams, lakes, and oceans and in their sediments. These humic substances may influence groundwater properties and are considered to play an important role in the geochemical cycle of organic carbon in aquatic systems. They are distributed in what they call dissolved organic matter (DOM) or dissolved organic carbon (DOC), which according to Aiken (1985) can be distinguished into two large groups, hydrophobic and hydrophilic groups. Each of the two groups can be subdivided again into the acidic, basic, and neutral subgroups. Humic substances form the bulk of the hydrophobic acidic fraction of the DOC, with concentrations reported to amount to 20 µg/L in groundwater and to ≥ 30 µg/L in surface water (Thurman and Malcolm, 1981). The DOC in water from different lakes in the United States is reported

by Steinberg and Muenster (1985) to contain 80% humic substances. The concentration is noticed to be 10 to 20 times greater in natural aquatic foam of rivers, lakes, and seas than in the water itself, and 90% (by weight) of the DOC in foam is humic matter (Mills et al., 1996). The presence of DOC or DOM often lends the water a yellow or darker color. Large amounts of dark brown, organic-rich water can be seen worldwide flowing from swamps and poorly drained areas into creeks and rivers, especially after a rainfall. Named at first *black water* by Tan et al. (1990), but renamed in this book *humic water* for reasons discussed in Chapter 1, Section 1.5.1.2, it is noticed flowing in the tributaries of the Amazon River in Brazil, where the Rio Negro is one of the most striking examples, and in the coastal streams of Sumatra, Kalimantan, and Papua, running between the vast expanses of peat deposits of Indonesia (Tan, 2008), to the rivers, lakes, and marshes in central Africa, Scandinavia, and the tundras in the arctic region. Many of the coastal plain streams and swamps of the southeastern United States carry also dark-colored water, attributed to the presence of organic substances in solution. As discussed in Chapter 1, Section 1.3.3, the organics were identified as *gelbstoff* (German for yellow material) or yellow organic acids. Judging from the names, they were then apparently not considered as important materials, but gradually the dissolved organic matter attracted a lot of research attention. Lately, DOM has become the subject of extensive investigations by many chemists, geochemists, and hydrologists, especially due to fear of creating a health hazard from the chlorination of drinking water containing colored substances (Bellar et al., 1974; Aiken et al., 1985). As a result of the analysis of samples from streams in Alaska, the Georgia–Florida border, Washington, and California, Lamar (1968) believed the yellow organic substances to be complex polymeric hydroxy carboxylic acids. According to Beck et al. (1974), the spectral characteristics of these complex acids closely resemble those of fulvic acids. More recent investigations by Tan et al. (1990) confirm the presence of humic matter as the reason for the dark color. As a result of further investigations using humic water samples of the Okefenokee Swamp, Satilla River, and Ochopee River in the Georgia coastal plain of the United States, Tan et al. (1991) show the composition of the humic matter to be characterized by a fulvic acid/humic acid ratio of 2.6. This means that fulvic acid is the dominant fraction, making up more than twice the concentration of humic acid in aquatic humic matter.

Mixed opinions exist on the nature of these aquatic humic substances. A number of authors believe that stream humic matter is similar in nature to its counterpart in soils (Stevenson, 1985; Beck et al., 1974; Shapiro, 1957;

Black and Christman, 1963). Their chemical properties are reported to be comparable to those of soil humic substances (Mayer, 1985; Steinberg and Muenster, 1985). However, other scientists are of the opinion that stream humic matter is different from soil humic matter (Malcolm, 1985). Another possibility is that some of the stream humic acid is of terrestrial origin, but after transfer into the streams and lakes has been subjected to further distinct changes in the anaerobic environment (Jackson, 1975). A number of data are present in the literature providing indications in support of the idea of such profound changes. For example, an investigation by Claus and Filip (1998) yielded results showing the biotransformation of humic substances in aquatic ecosystems by laccase-producing fungi *Cladosporium cladosporioides* and *Polyporus versicolor*. The fungi, isolated from the water of a peat bog lake, are noted to be capable of cleaving humic substances, altering them from being highly to considerably less aromatic in structure. The humic substances are reported to become more aliphatic, exhibiting core structures composed mostly of carbohydrates and amino acids only. The present author expects that, no doubt, many other phenoloxidase-forming microorganisms may also exist in the aquatic environments with high lignolytic activity, enabling the degradation of lignin components in humic compounds. The latter will then result in structural changes, leaving the distinctive "stamp" or markings of aquatic systems in the affected humic substances.

All the issues above have apparently necessitated the creation of a division into two broad categories of humic fractions in water, an *autochthonous* and an *allochthonous* variety of aquatic humic matter. The autochthonous types are formed from indigenous aquatic organisms, and as indicated above exhibit both low lignin contents and aromatic structures, but are considerably higher in carbohydrate and aliphatic structures. On the other hand, the allochthonous variety originates from the soil or better more from vascular plants rich in lignin, hence is characterized by substantially higher aromaticity and lignin content. Such a division is especially important for humic matter in lagoons, bays, and other lake or marine environments where substantial amounts of plankton, algae, and kelp can grow. It is believed that in big lakes and oceans, most of the humic matter is autochthonous and only on the beaches or coastal zones and in the lagoons or estuaries is there a mixture with soil humic matter. In the ocean remote from any terrestrial influence, the gelbstoff mentioned earlier is assumed to be formed from dead phytoplankton (Kalle, 1966). The supposition above finds support from the investigations by McKnight et al. (1991, 1994) on aquatic fulvic

acids in microbially based ecosystems of lake waters and in algal-rich ponds, both in Antarctica, which are considered not to be affected by terrestrial or allochthonous influences. The fulvic acid originates, allegedly, solely from remains of bacteria and algae that have grown under the protection of a continuous ice cover. This fulvic fraction is reported to be characterized by low aromaticity and low C/N ratios ranging from 9 to 15. Ishiwatari (1985) indicates that humic matter derived from phytoplankton cells will undergo diagenetic changes when deposited at lake bottoms. The deeper it is buried in the lake bottom sediments, the greater tends to be the decrease over time in humic acid and fulvic acid contents, which is generally accompanied by a corresponding increase in the amount of humin. Differences in aquatic systems have also been cited recently to yield different types of aquatic humic matter. Humic substances from dystrophic environments are believed to be more aromatic than those from eutrophic and mesotrophic systems (Klavins and Apsite, 1997).

4.2.4 Humic Matter in Geologic Deposits

Humic matter also occurs in the vast geologic deposits, such as lignite, coal, oil shale, and fossil fuel. Extensive deposits of lignite in the United States are located in Utah, North Dakota, Idaho, New Mexico, and Texas. In other parts of the world, lignite is also found in Germany, Poland, Serbia, Greece, and Russia, where it is generally considered the lowest rank of coal with characteristics between butinous or soft brown coal and peat. The lignite in North Dakota is often called *leonardite*, after A.G. Leonard, the first director of the U.S. Geological Survey in North Dakota. It is believed to be an oxidation product of lignite, associated with surface mining. High-quality-grade lignite and/or leonardite may contain 80% to 90% humic matter. It forms at present the major source for commercial production of humic acids and humates for use as soil amendments and a variety of other industrial purposes. Although commercial-grade lignite is, on average, composed of 60% to 70% humic matter, contents of 30% to 60% have been reported occasionally (Jackson et al., 2008; Stevenson, 1986). Other types of geological materials that have attracted increasing attention at present from especially the industry section are kerogen, bitumen, and mumijo.

Kerogen is defined by geologists as a mixture of organic matter in sedimentary rocks (Tucker, 1988). In Chapter 1 (Section 1.3.2), mention is also made of its occurrence in intergalactic dust. The name kerogen was allegedly introduced in 1912 by Alexander Crum Brown, an organic chemist from

Scotland. The soluble fraction of kerogen is called *bitumen*, which is also known as *asphalt*. Kerogen containing shale is also often called *oil shale*. However, several geochemists and soil scientists consider kerogen to be a condensed form of aquatic humic acid, solidified through *diagenesis* into a geologic deposit similar to sedimentary rocks (Hatcher et al., 1981a). Some credible support for this idea was provided earlier by Jackson (1973), who showed from his studies on humic matter extracted from Precambrian bitumen the relation of humic formation from pre-Paleozoic algae.

Shilajit, salajeet, mumijo, or mumie is another geologic deposit allegedly also rich in humic and fulvic acids. The name shilajit is from Sanskrit, which means rock-invincible, perhaps because it becomes visible when it appears as mineral oil seeping from cracks in the mountain rocks due to heating by the sun. It is the common name in India used for a black asphalt-like deposit in the Altai and Tibet mountains of the Himalayas, the Caucasus Mountains of central Asia, and the Gilgit-balistan mountains in Pakistan. The Caucasian deposits are known in Russia more by the name of *mumijo* or *moomijo* (from Greek). Similar deposits have also been reported in the Antarctica region. The Shilajit-mumijo material has attracted considerable attention from the pharmaceutical industry for its use in alternative medicines as anti-inflammatory agents (in arthritis), anti-ulcer, anti-stress, anti-anxiety, anti-aging, and in general as “wonder drugs” (Winston and Maimes, 2007). These claims have been investigated and are still under investigation by scientists in India, Germany, and Russia as being caused by the humic contents in the material (Schepetkin et al., 2003; Klöcking, 1994a, b).

As discussed in the aforementioned sections, these geologic deposits rich in humic matter have been formed in time from bog, peat, and muck. The transformation process is called *diagenesis* by geochemists (Hatcher et al., 1985), but in soil science such a transformation is called *metamorphism*, since high pressures and temperatures are required to induce the needed compaction and drastic chemical changes (Miller and Gardiner, 1998; Tan, 2000). During the conversion of peat into coal, it is believed that humic acid is undergoing drastic changes. The theory presented by Van Krevelen (1963) and Stach (1975) assumes that humic acid is converted into humin by a condensation process during the transformation of peat into lignite and subbituminous coal. In soil science and humic acid chemistry, the reaction is called *polymerization*, instead of condensation. Today, proponents of supramolecular chemistry may incline to impose the self-assembling process for such a transformation, but would then have to contend with humin being more thermodynamically stable than humic acids. This seems likely to be

the case judging from the amount of humin noted to increase during further *coalification* processes into bituminous coal, anthracite, and finally graphite as the stable end product. Breger (1963) and Hatcher et al. (1985) recognize two types of coal derived from peat and peat bogs: (1) *humic coal* formed from peat containing humic substances derived from vascular plants, and (2) *sapropelic coal*, formed from peat derived from sapropel composed of algae remains.

The geochemical condensation and coalification theory seems to suggest that lignite and coal deposits are then rich in humin. Although Hatcher et al. (1985) admitted that more data are required to confirm it, these authors indicate that with increasing coalification, the material is more likely to become less extractable with dilute alkali solutions. They expected less humic acid and fulvic acid to be extracted with increasing rank from peat, lignite, to coal. However, more recent investigations show this to be true for coal, but are unable to confirm the contention above for lignite. As a result of analysis with commercial sources of humates, Lobartini et al. (1992) show a commercial-grade lignite from North Dakota to be composed of substantial amounts of humic acids. The humic acid content is 99.0% in contrast to that of fulvic acid, which amounts to 1.0% only. Earlier investigations yielded similar results. Lignite or leonardite samples from eight different locations in Utah and North Dakota were also noted on the average to be composed of 99.5% humic acid and 0.5% fulvic acid (Tan and Rema, 1992, 1993). At the present stage of knowledge, the reason for such low contents of fulvic acids is not known yet. While awaiting more information, it can perhaps be speculated that most of the fulvic acids, if not all, have been polymerized into humic acids, whereas the remainder was lost by leaching during the time of deposition.

For completeness, the theory embraced by geochemists that humic matter is present in substantial amounts in a variety of prehistoric sedimentary rocks should perhaps also be mentioned. Precambrian (approximately 4700 million years ago), lower and upper Paleozoic, and Mesozoic to Cenozoic (recent life, today) rocks are considered to contain humic acids (Swain, 1975). Swain believes that organic soils may have become widespread by the appearance of large terrestrial plants with extensive root systems in those prehistoric days. The increased occurrences of petroleum and coal deposits during the upper Paleozoic period have been used by the author above as markers or tracers for organic matter production and deposition in the past. This type of humic matter can be called geologic humic matter, as suggested by Stevenson (1994); however, in the opinion of the present author, it should better be called *paleontologic humic matter*.

4.2.5 Humic Matter in Agricultural, Industrial, and Municipal Waste

Agricultural, industrial, and other operations in our modern society produce large amounts of waste, some inorganic and some organic. The soil is traditionally the site for disposal of all these wastes. People have been discarding waste since prehistoric times, but there was little concern in the old days about pollution and degradation of the environment because the human population was still small and there was plenty of space on Earth for the amount of waste produced. However, with population growth and the revolutions in industry and agriculture, huge amounts of waste and a variety of new types of pollutants have been produced. Because of concern about contamination and especially the degradation in environmental quality starting in the 1970s, a number of waste disposal methods were explored. Today, waste is buried in landfills or dumped in the sea, whereas the organic part is incinerated or used again on farmlands. When waste is used again as soil amendment or for any other useful purpose, it is no longer considered waste but rather a valuable resource material (Tan, 2000). This is the case today with poultry litter, sewage sludge, and compost. The poultry industry in the southeastern United States is estimated to produce 11 million tons of litter each year (Tan et al., 1975). In the state of Georgia alone, the official 1975 assessment amounted to a production of 720,000 metric tons of manure at the time of removal from the point of production of broilers. To this amount should be added 450,000 metric tons of commercial hen manure, 79,200 tons of breeders manure, and 100,000 tons of turkey manure (Muller, 1975). The ever-expanding poultry industry, resulting in more increases in manure, may result in sanitary disposal problems, although part has found application as soil amendments (Campbell, 1973). Aside from being an organic fertilizer, poultry manure is a valuable source of humic matter. The water-soluble fraction has been identified to carry properties similar to those of fulvic acids (Tan et al., 1971a, 1975). Owing to the huge amounts of poultry manure available each year, its potential as a humic acid source is comparable to that of lignite or peat deposits. Not much information is available on this matter today.

Another type of waste containing humic matter is sewage sludge, produced in amounts even more enormous than poultry manure. The figures reported by Larson and Schuman (1977) of 5680 m³ of sewage sludge barged daily from Chicago, Illinois, to its dumping site, and 5450 m³ of raw sludge transported daily by gravity through open ditches to landfills in Melbourne, Australia, demonstrate the colossal dimension of production. Sludge, as a

reservoir of humic substances, attracted considerable research attention during the period 1970–1980. The NaOH extract yields substances with properties similar to those of humic and fulvic acids (Tan et al., 1971a). The fulvic acid fraction was the subject of extensive investigations for its metal-chelating power by Sposito et al. (1978, 1981). Senesi and Sposito (1984) regarded sludge-derived fulvic acid equivalent in its complexing capacity to soil fulvic acid.

Other examples of human-made wastes containing humic matter are compost and deposits of cattle manure in feedlots. The so-called CAFOS, for confined-animal feeding operations, in Texas are notorious for animal-waste production, creating annually an estimated 120 million metric tons of manure (Ivins, 2001). These huge amounts of animal wastes, not confined only to cattle manure but also including hog and chicken manure, are perhaps sources for fulvic acids that being soluble are reported fouling the water supply in the Lake Waco area, the Playa lakes in the high plains, and east Texas. Sitting right on top of the recharge zones of the Paluxy, Trinity, and Ogallala aquifers, these deposits are too close for comfort. Waste from food-processing plants is an additional source of humic substances. Especially beer breweries yield large amounts of residues that after fermentation and decomposition become potential sources of humic and fulvic acids. Wastes from canning industries are not important as a source of humic substances since most of them are too valuable and can be recycled into animal feed or into fertilizers. Finally, the thick dirty black material burdening heavily polluted drainage ditches, rivers, and lakes, which can be noticed in developing as well as in developed countries, should be mentioned. This often foul-smelling black mass is an often forgotten source of humic matter. Unfortunately, not much is known yet on the humic matter contained in man-made wastes.

4.3 Classification of Humic Matter

From the preceding sections, it is perhaps possible to recognize five general groups of humic matter, i.e., soil humic matter, aquatic humic matter, humic matter from wetlands, geologic humic matter, and anthropogenic humic matter.

4.3.1 *Terrestrial or Terrigenous Humic Matter*

This was explained earlier as the group of humic matter in soils, composed of substantial amounts of humic acid and fulvic acids. According to the

ligno-protein theory, the humic substances are mainly ligno-protein complexes. Lignin, considered as the fundamental constituent of these humic substances, can be distinguished into three major types on the basis of its monomers: (1) softwood lignin, characterized by coniferyl alcohol, derived from softwood or coniferous plants (gymnosperm); (2) hardwood lignin, characterized by sinapyl alcohol, common in hardwood (dicotyledonous angiosperm) vegetation; and (3) grass or bamboo lignin, characterized by coumaryl alcohol, common in grasses and bamboo (monocotyledonous angiosperm). Consequently, the suggestion is presented for a possible division of soil humic matter into three subgroups (Tan, 1998):

Softwood soil humic matter—This type of humic matter is formed from softwood lignin monomers, and is structurally characterized by coniferyl alcohol.

Hardwood soil humic matter—This is humic matter made up mostly of hardwood lignin monomers, and structurally characterized by sinapyl alcohol.

Grass or bamboo soil humic matter—This is the humic matter formed from grass or bamboo lignin, and structurally characterized by coumaryl alcohol.

It should perhaps be realized that a sharp distinction among the three subgroups above can only be established in soil humic matter affected by monocultural environments. In nature, it is common to have a hardwood forest mixed with a conifer stand and an underbrush vegetation of grasses and bamboos. In such a complex ecosystem, the humic matter will likely be more of a mixture of the three groups above. The idea is presented here to promote more research on the structure of humic substances. The identification of the type of aromatic nucleus of the so-called humic acid monomers may shed light on the viability of the proposed division of humic matter into the three groups as stated above.

4.3.2 Aquatic Humic Matter

This is the humic matter in streams, lakes, and oceans, and their sediments. The term kerogen is sometimes used for humic matter in aquatic sediments (Swain, 1975). However, Mayer (1985) considers it to be a condensed form of aquatic humic acids, produced by diagenesis of the latter (Hatcher et al., 1985).

In the discussions above, it is indicated that fulvic acid is the dominant substance in the humic matter carried by water, whereas humic acid is only a minor constituent. However, humic acid may be present in appreciable amounts in the humic matter deposited at lake or sea bottoms. On the basis of origin, two groups of aquatic humic matter have been recognized:

Allochthonous aquatic humic matter—This is humic matter brought from the outside into the aquatic environment (water). The humic matter is formed in soils and after formation leached or eroded into rivers, lakes, and oceans. Although physical and chemical changes may have been induced by the aquatic system, the nature of the humic matter is still related to soil (terrestrial) humic matter.

Autochthonous aquatic humic matter—This is humic matter formed in the aquatic environment from cellular constituents of indigenous aquatic organisms. In marine sediments, this kind of humic matter consists of carbohydrate–protein complexes (Degens and Mopper, 1975; Jackson, 1975). Sapropel, copropel, and the like can perhaps be grouped in this category.

4.3.3 Wetland or Peat Humic Matter

This is humic matter derived from material formed in poorly drained ecosystems accumulating as thick deposits of bog, peat, and mucks. It is composed of fulvic acid and humic acid, with the humic acid content reported to increase from peat to muck. Humin was also mentioned earlier as a possible fraction present in substantial amounts. At the present stage of knowledge, it is not known whether the properties of the humic substances may be different in the different kinds of peat, since peat from sphagnum is chemically different from peat of heath vegetation or peat formed from woody trees. In peat deposits, formed in systems resembling the soil ecosystem except for the poor drainage, the humic acid is reported to exhibit properties closely related to those of terrestrial humic acid. Peat humic acid is believed to have properties similar to brown humic acid, although some are skeptical about this. With future developments in technique and instruments, differences can perhaps be detected between humic matter formed under well-drained and poorly drained systems. The properties of humic acid in sapropelic peat are assumed to be far remote from those of its soil counterpart.

4.3.4 Geologic Humic Matter

This is the humic matter in lignite or leonardite and the various types of coal. It is composed mostly of humic acids, although many believe that it also contains a lot of humin. Because of the aging process, most of the fulvic acids have been squeezed and polymerized into humic acids by diagenesis reactions. Environmental processes, such as leaching, may have assisted by decreasing the fulvic acid content further. A subdivision into geologic and paleontologic humic matter can perhaps be made on the basis of the geologic ages of the deposits.

4.3.5 Anthropogenic Humic Matter

This is the humic matter derived from agricultural, industrial, and domestic wastes, and from material in polluted waterways. From the scant data available, it is expected that this type of humic matter is composed of fulvic acid and humic acid. In the polluted drainage canals and ditches, the water is often yellowish to brownish, suggesting perhaps the presence of large amounts of fulvic acids. Not much is known yet about these anthropogenic humic substances, although at one time poultry manure and sludge-derived fulvic acids are considered comparable to soil fulvic acid.

Chapter 5

Genesis of Humic Matter

5.1 Major Pathways of Humification

5.1.1 Humification versus Diagenesis

The process by which humic matter is formed has been called in soil science *humification*, which involves a number of biochemical reactions. Several versions of biochemical reactions are present, recognized as different pathways in the condensation–polymer concept, and with the advancing knowledge in the twenty-first century several more new pathways have been added on the basis of the self-assemblage concepts. Traditionally a subject in soil science, humic matter is now also a field of study in other scientific disciplines, e.g., geology, hydrology, and limnology. Such an expansion into different directions has brought new ideas and concepts, undoubtedly enriching the knowledge of humic substances. Terms used in geology are now also applied in humic acid science, and among them, the term *diagenesis* seems to be applied quite often to refer to the formation of humic substances, as employed in particular by geologists, geochemists, hydrologists, limnologists, and some chemists (McSween et al., 2003; Hedges and Keil, 1999; Hatcher et al., 1985). However, the term has the connotations of geological processes related closely to consolidation and *lithification* of unconsolidated material into “solid” rocks. In pedology, soils are unconsolidated materials weathered from rocks, and humic matter is synthesized during soil formation as one of the major constituents essential in the creation of soils. The humic substances are also closely connected to the organic and nitrogen cycles in the environment, and it is perhaps questionable to assume the two cycles above to be geological processes, although today many soil,

environmental, and chemical reactions seem to be readily claimed as belonging to the domain of geology by using the term *biogeochemistry*. This has, of course, created a lot of controversies in soil science as has the case of soil chemistry versus geochemistry (Tan, 2011). The issues and advantages of diagenesis in humic acid chemistry will be examined in more detail in the following section.

5.1.1.1 Issue of Diagenesis in Humification

Diagenesis is a term used originally in geology to describe the chemical, physical, and biological changes of inorganic and organic sediments, involving lithification after their deposition and burial (Horsfield and Rullkötter, 1994). The name lithification, derived from the Greek word *lithos* (= rock), refers to a process of compaction or consolidation into solid rocks and in particular sedimentary rocks. The terms above have filtered from geology into soil science to describe the formation of humic substances, as is the case with many other geological names and ideas due to the increased research in humic matter by geologists and related scientists. Although, as indicated above, the imported geologic theories have, no doubt, enriched the concepts in soil science, they have unfortunately also brought a lot of controversies and confusion. Geologists and geochemists suggest that diagenesis of organic matter composed of plant biopolymers produces a condensed form of organic residue that they call *geopolymer* (McSween et al., 2003). Since a precise definition is not given, the concept of geopolymers is rather vague and can be interpreted at will. After introducing the term geopolymer, McSween and coworkers continue with reporting that in soil ecosystems diagenesis yields humin, whereas in swamps the process produces lignite and/or coal. In marine and lacustrine systems, the authors believe diagenesis of organic residue to result in *kerogen*, a geopolymer. The common knowledge is that kerogen is generally formed from humin and, in soil science as well as in geology, considered a condensed form of humin. The difference is that in soil science, humin is known to be composed of degraded plant biopolymers. The question is now raised about what the differences are between plant biopolymers and geopolymers. Another issue is that although several geologists seem to support McSween's opinion of the aquatic origin of kerogen, many other geochemists also note the existence of kerogen from terrestrial systems. Besides the presence of sapropelic and planktonic kerogen, the two distinct types originated from aquatic organisms, humic and residue kerogen are also present. The latter two are more

aromatic in nature owing to their origin from coastal (terrestrial) vegetation rich in lignin and terpenes. On the other hand, the wet conditions as prevailing in marshes, swamps, and wetlands are generally assumed to be conducive for the formation of peat, which in the early stages of diagenesis can be converted into lignite. Apparently during the later stages of diagenesis, burial of lignite causing increased condensation results in the transformation of lignite into brown coal. The middle to late stage of diagenesis is called *catagenesis*, whereas the last stage is known as *metagenesis* by which the organic matter deposit is broken down into hydrocarbons along with methane and other natural gases by a process commonly called “cracking.” Because metagenesis occurs at elevated temperatures from 120°C to 200°C (302–392°F), a number of geologists consider the process to be similar to metamorphism.

5.1.2 Humification in Relation to Carbon Sink Formation

The process of humic formation is often also considered a form of accumulation and storage of carbon-containing compounds. Humus and humic matter may therefore qualify to be regarded as a natural carbon sink next to the oceans and terrestrial plants. Several scientists even claim that soil humus, including its humic components, represents a carbon storage medium with a carbon content significantly higher than terrestrial vegetation combined together with the atmosphere (Swift, 2001; Batjes, 1996) or when compared with glomalin as discussed in Chapter 2, Section 2.4. It is perhaps clear and understandable by now that plant litter and other biomass materials accumulate as organic matter in soils. They are then subjected to decomposition by chemical weathering and biological degradation, resulting in the more resistant organic polymers, such as lignin, waxes, terpenoids, and many others as discussed in Chapter 2, Sections 2.2 to 2.3, being left behind as a valuable carbon-rich residue in the form of humus and humic substances. In this way, organic matter tends to accumulate in litter and humus of soils from the cold regions under the boreal forests of North America and the Taiga forests of Russia and Siberia to the soils in the humid tropics under the thick expanses of tropical rainforests. Grassland soils of the semihumid regions in the world and peat soils of the wetlands also contribute to accumulation and storage of organic carbon. As indicated above, in the form of soil humus and humic substances, the huge deposits of accumulated organic matter then function as a very important carbon sink. All of the above will be discussed again in more detail in the following sections.

5.1.3 Issue of Biopolymer Degradation and Abiotic Condensation Pathways

Although some people are of the opinion that the mechanisms for synthesis of humic substances are not clear, a number of hypotheses have in fact been presented on the processes of formation of humic matter, such as the biopolymer and the recently introduced self-assemblage pathways. All agree that the materials for formation originate mostly from plant materials, although in practice animal residue can also be transformed into humic matter. In the conventional biopolymer concept, two different pathways seem to exist, which vary in the way the sources of original or raw materials are utilized in the synthesis of humic substances. Whereas one version, the older established version, favors one course of reactions involving polymerization of small organic fragments and molecules into humic matter, another version, suggested by Hedges (1988), recognizes two different routes. In the first route or step, Hedges envisages slight alterations in the original biopolymers to cause the direct formation of humic substances. This route, called *biopolymer degradation* (BD) by Hedges, assumes that the biopolymers in plants, degraded by slight changes, are transformed immediately into humic acids, which decompose via fulvic acids into labile small components. Part of these small molecules are subject to mineralization or loss by leaching, whereas another part are used for reconstruction into humic acids by a second route of reactions, called *abiotic condensation* (AC). Hedges' BD and AC concepts are based on different rationales because of his idea that humic acid is destroyed and later reconstructed (see Hedges, 1988, p. 47, figure 1). The concept of abiotic condensation used by Hedges also appears to deviate from the principles of condensation reactions. In polymer chemistry, condensation-polymerization means that H_2O is lost during polymerization (condensation). In addition, Hedges' AB concept also differs from the standard opinion of abiotic condensation (see also Hayes and Malcolm, 2001). These issues will be examined in more detail below.

In the older established biopolymer or polymerization concept, it is unusual to consider a slightly weathered broken lignin particle as a humic acid molecule, whereas the logic for the destruction and later reconstruction of humic acids raises serious questions. Instead, the older theory claims and underscores the idea that plant biopolymers are decomposed first into their monomers and other smaller organic components. Humic substances are then formed by interactions between these small organic fragments, followed by condensation-polymerization (Kononova, 1961; Tan, 2011). This

theory suggests fulvic acid to be formed first, which is then transformed into humic acids. The polyphenol or phenol, quinone, and sugar–amine condensation theories belong to this category of the polymerization concept. The name *abiotic condensation process* has also been employed for this polymerization pathway. The ligno–protein theory of Flaig (1988), used as an example by Hedges in support of his AC concept, focuses on the breakdown of lignin and further oxidation of the degradation units into quinone derivatives. Flaig's idea is, therefore, an additional excellent example supporting the polymerization or the original abiotic condensation theory. Hedges' (1988) proposal of an abiotic condensation pathway in the synthesis of humic acids is a model based on different principles. Condensation is confusingly defined by Hedges as repolymerization of humic acids from small organic molecules generated by destruction of humic acids. He considers abiotic condensation (AC) to be a constructive follow-up pathway of his biodegradation (BD) steps (see Hedges, 1988, p. 48).

Hayes and Malcolm (2001) believe that the rate of depolymerization apparently depends on the free oxygen content, and humification will be retarded in anaerobic conditions. It is true that a lot of oxygen is required for oxidation reactions, but the issue can be raised whether a lack of free oxygen—in the presence of bound oxygen, such as Fe_2O_3 , MnO_2 , and others—will severely inhibit the humification process. As discussed in Chapter 2, huge deposits of peat and bogs, rich in humic matter, are instead formed in wetlands, marshes, and swamps, where anaerobic conditions prevail. In addition, significant amounts of aquatic humic substances are formed in anaerobic conditions where oxidation processes are at a minimum. This is particularly true for the formation of the autochthonous humic fraction, but even for the allochthonous aquatic humic part. It was discussed in Chapter 4 that the allochthonous character can be changed by the aqueous environment yielding humic products exhibiting distinctive brands of the anaerobic effect.

5.1.4 Relation of Modern Pathways to the Polymer Concept

As discussed in the preceding chapters and above, many scientists tend to believe the biopolymer or polymerization theory to be outdated because of the appearance of the recently introduced self-assemblage ideas poised to replace it. In the author's opinion, the old established theory on humification is neither outdated nor obsolete. Many of its concepts are compatible for application with the micellar and nanotube membrane concepts. The older

concept consists in fact of several different pathways, such as the ligno-protein and quinone, polyphenol or phenol, melanoidin or sugar–amine, and polyunsaturated lipid pathways (Flaig et al., 1975; Schnitzer and Khan, 1972; Ziechmann, 1994; Stevenson, 1994; Harvey and Boran, 1985; Tan, 2011). The degradation products of lignin, protein, amino acids, polyphenols, lipids, and carbohydrates—as straightforward broken chemical species or in combination—used in the various polymerization pathways can also be applied in the self-assemblage process. Instead of attraction by the usual strong bonds, such as covalent bonding and esterification common in polymerization, the principles in supramolecular chemistry allow the same components to self-assemble by weak chemical bonds into aggregates with the most stable thermodynamic arrangement. The two major pathways of humification are illustrated in Figure 5.1, with the top picture showing the

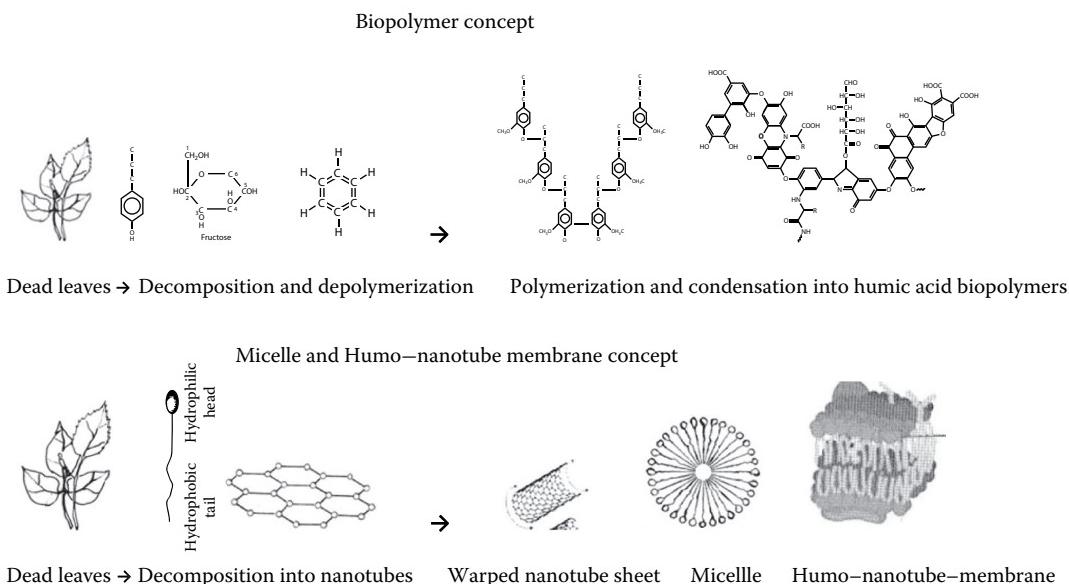


Figure 5.1 Diagram of humification pathways. (Top) Biopolymer or polymerization concept illustrating direct condensation–polymerization of small decomposed organic fragments into humic substances. (Bottom) Micellar and nanotube membrane concepts showing formation of micelles and membranes. The micelle shown is of the spherical type and looks like a bicycle wheel supported by spokes or radiating bars, which are in fact the amphiphilic components. The size of the micelle is determined by its radius, which equals the length of the amphiphile. In the nanotube concept, it is 2–10 nm. The number of amphiphiles determines the mass of the micelle. The membrane illustrated is a bilayer of rectangular micelles and looks like a sandwich. Its thickness is two times the length of the amphiphile. Adapted in part from www.assistancescolaire.com; accessed February 21, 2013.)

conventional biopolymer concept, and the bottom the self-assembling supramolecular pathway. As originally explained by Wershaw in 1986 and later confirmed by his second publication (Wershaw, 1999), decomposition of plant residue provided the great mixture of small biopolymer fragments at various degrees of decomposition. Some of them are in the forms of amphiphiles, which in Figure 5.1 aggregate to form micelles and/or bilayers of micelles, called membranes by Wershaw. These micelles and membranes are Wershaw's models of humic substances. The micellar theory above is later refuted by Piccolo (2002), who suggests a process of random assembling yielding structureless humic substances. However, electron microscopy by Tan (1985, 2011a, b) shows evidence of well-ordered structures of the humic substances, supporting Wershaw's model. This discovery is also one of the reasons for extending the principles of supramolecular chemistry at nanolevels (Tan, 2011b). As indicated in Chapter 4, Tan (2011b) assumes that during the breakdown of organic matter into smaller fragments, a lot of submicroscopic particles are also produced, varying widely in size and length from nanometers to micrometers. These nanoparticles, expected to be capable of self-assembling by the process earlier called *nanofabrication*, seem to create supramolecular associations of humic substances with distinct and well-ordered identifiable molecular structures. The presence of peptides and other amphiphilic nanofibers is needed in the formation of membranes. Together with carbon nanotubes, they are essential in the creation of humic substances. The hexagonal arrangements of carbon in the carbon nanotubes—closely resembling benzene and phenol structures—provide the aromatic carbon structures, whereas the peptide nanofibers and membranes satisfy the nitrogen requirements for the synthesis of humic substances. This concept seems closely related to the conventional ligno-protein or biopolymer theory. The difference is that in the humo–nanotube membrane concept suggested by the present author, the humic molecules are produced by self-assembling involving weak chemical bonds holding the molecules together. In the process, some repolymerization of the small broken fragments may perhaps take place. Evidence for the humo–nanotube associations in natural ecosystems has been presented by Tan (1985, 2011a, b). The sizes of the humic micelles can be estimated from the sizes of the component amphiphiles. In Wershaw's concept, the size of a spherical (circular) micelle is then determined by its radius (Figure 5.1, bottom diagram), which equals the length of an amphiphile. In the humo–nanotube concept of the present author, the size of the micelle is much smaller but can be estimated with better precision since its radius generally equals the length

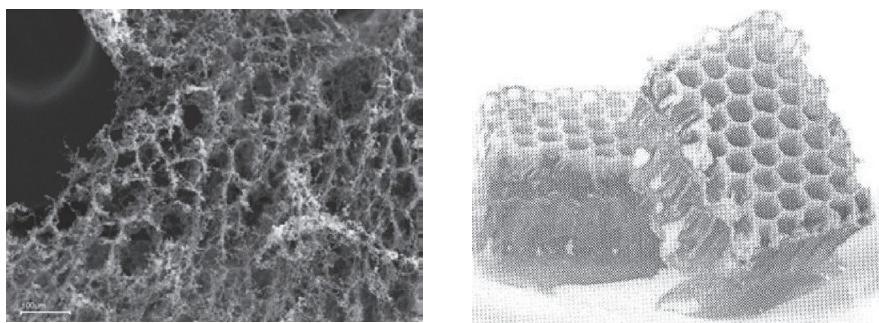


Figure 5.2 Scanning electron micrograph (left) of humic acid extracted from lignite (Tan, 2011b) showing the characteristic nanotube membrane structures resembling a beehive honeycomb as shown on right.

of a nano-amphiphile, which is in the order of 2–10 nm. The number of amphiphiles determines the mass of the micelle, which then provides clues for determination of molecular weights. The thickness of the membrane (in Figure 5.1, bottom) is two times the length of the amphiphile. In his most recent analyses of humic acids, the presence of the distinctive honeycomb pattern is clearly demonstrated by Tan (2011b) by scanning electron microscopy (Figure 5.2). Such a structure, called by Tan *humo-nanotube membrane structure*, is presumably the most stable thermodynamic association that can be attained by self-assembly of humic acids. Such an orderly structural pattern relates better than Piccolo's random bundles of humic molecules in explaining issues of consistent carbon and nitrogen contents and reproducible spectroscopic features of humic substances published in various journals.

5.2 Precursors of Humic Matter

The plant biopolymers of importance in humic matter synthesis are, for convenience, called here precursors of humic substances. The major components of higher plants important as sources for the formation of humic matter are lignin, cellulose, and hemicellulose, called polysaccharides, lipids, proteins, and many more. Phenols, polyphenols, quinones, and amino sugars synthesized by microorganisms have recently also been recognized as important raw materials for the synthesis of humic substances. Since degradation of lignin can also produce phenols, two sources of phenolic compounds can be distinguished in soils. All these compounds, present

originally in the form of large molecules in the plant tissue and soil, have been discussed in detail in Chapter 2, Section 2.2, as the nonhumic fraction of soil humus. It was presented earlier to show their chemistry, because as stated before, many people often wonder, if not become confused about, what biopolymers are, what aromatics mean, and what the difference is between phenol and quinone. A number of hardcore scientists even argue about the correctness of using terms such as phenolic-OH and the like. It sounds like basic organic biochemistry but some of the basic definitions are needed to explain the chemical behavior of the compounds, necessary in understanding their interaction and/or reactions in humic acid chemistry.

The biopolymers, called humic precursors here, are produced mostly through photosynthesis by terrestrial and aquatic plants. As explained above, they are commonly believed to be broken down first before being used in the synthesis of humic substances.

5.3 Theories of Humification

It is perhaps apparent from the discussions in the preceding sections that a variety of organic compounds take part in the humification processes. In fact, not a single organic compound can theoretically be ignored, and a variety of substances is expected to react in a variety of combinations, which is therefore creating all the consternations about humic acids and fulvic acids being artifacts or operational compounds and the like. The biopolymers produced by plants, when released into the soil upon decomposition of the plant tissue, can be used as such or in slightly to highly degraded forms to form humic substances. Lignin or degraded lignin may react with decomposed protein or peptide polymers and other large organic polymers, e.g., polysaccharides and phenol polymers. This type of humification is reflected by the ligno-protein theory. A second group of theories argues that the large plant biopolymers are decomposed first into their monomers or smaller molecular compounds before humification. According to this idea, single molecules of phenols, quinones, monomeric amino acids, and ions, such as NH_3^+ , are considered the basic molecules instead of lignin; hence, names such as the polyphenol or phenol theory have been advanced for this type of humic matter synthesis. The reaction between the small molecules can take place abiotically, meaning purely chemically; however, it should be realized that the phenols, quinones, and monomeric amino acids have been derived biologically. Not only can they be formed from the degradation of

large biopolymers such as lignin, but they can also originate from metabolites of microorganisms. In addition to the ligno–protein and phenol theories above, the *sugar–amino condensation theory* is perhaps a third theory of major importance. It is sometimes called by different names, such as the *melanoidin pathway* or *Maillard reaction*. Sugar and amino acid form the starting points, instead of lignin or phenol and quinone, as is the case in the ligno–protein and phenol–protein theories, respectively. These are the three major older concepts on the synthesis of humic matter and as discussed above are not obsolete and can be applied in conjunction with the newer concept based on the principles of supramolecular chemistry. These theories on humification will be examined below in some more detail.

5.3.1 Ligno–Protein Theory

This is the theory that has dominated, for years, the view of many scientists on the synthesis of humic matter. It has been promoted by renowned scientists in the subject worldwide, such as Waksman (1932) in the United States, Kononova (1961) in Russia, and Flaig (1975) in Germany. The concept is called the *lignin theory* by Stevenson (1994), although the author failed to state his reason for this. Normally, lignin and amino acids (protein) are considered to form the core of humic acid, and not lignin alone. Perhaps it is because the synthesis of humic matter does not only involve reactions of lignin and protein but also reactions between lignin derivatives and simple nitrogenous compounds, such as NH₃, which is not a protein. Stevenson is also of the opinion that this ligno–protein or lignin theory is currently obsolete and has been replaced by the polyphenol theory. This opinion is, of course, subject to debate, and the two theories can perfectly coexist. In addition, the idea that lignin and protein derivatives play an important role in the synthesis of humic substances has recently gained considerable support. Using advanced techniques of ¹³C nuclear magnetic resonance and thermochemolysis–gas chromatography/mass spectrometry, Chefetz et al. (2002) have discovered large amounts of lignin, protein, and cuticular material in the structures of their humic acid specimens.

The ligno–protein theory envisages lignin units entering into reactions with amino acids forming the core of the humic molecule, hence the name *lignin* and *protein* or the ligno–protein concept. To this core, a variety of other organic substances, e.g., polysaccharides, can be attached. A simple illustration of the author's idea of these reactions is provided in Figure 5.3. For simplicity, the lignin fraction is represented by coniferyl alcohol, whereas

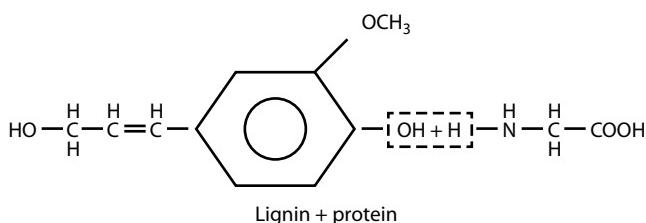


Figure 5.3 Schematic formation of a humic molecule according to the author's ligno-protein concept. The lignin monomer is a 4-hydroxy-3-methoxy phenyl propane or commonly known as softwood lignin.

the protein fraction is shown by a monomeric amino acid. The reaction product formed should be regarded as a prototype, or humic-like substance. Addition of COOH and OH groups to the aromatic core is required for the development of the smallest possible monomer of a humic molecule. According to Flaig's theory, the propane (= lignin) section is oxidized to yield by carboxylation a COOH group, whereas the methyl group can be converted into an OH group by hydroxylation, producing in this way the phenolic-OH group. The monomers, as noted in Figure 5.3 reacting together as such, are expected to form the smallest possible molecule of a humic substance. The smallest humic substance known today is fulvic acid, which upon polymerization can be converted into humic acid. Further polymerization or condensation will form humin and ultimately kerogen, which constitute the end products of the reactions. However, recently, a slightly different version has been presented on the diagenetic transformation of the humic substances, which clouds the issue on biopolymer degradation and polymerization processes in the synthesis of humic substances. Stevenson (1994) is of the opinion that lignin will yield humic acid, which can be directly transformed into coal. Stevenson's diagram, given in Figure 5.4 for the sole purpose of explaining the confusion it creates, suggests that formation of

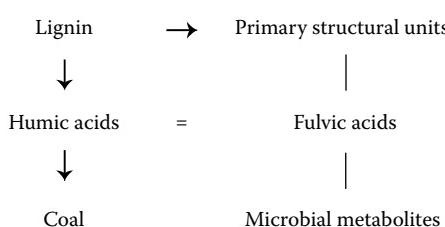


Figure 5.4 Diagenetic changes of humic matter according to Stevenson (1994).

humic acid directly from lignin is a biopolymer degradation process, but the transformation of humic acid into coal is a condensation process. Stevenson is of the opinion that a biopolymer degradation process starting with lignin to humic acids will end with the formation of fulvic acid. No indications are provided that the latter can also be broken down further into even smaller units. In this process, the fulvic acids are assumed to be created only as by-products since the main route is from lignin → humic acids → coal, as also indicated by the arrows in Figure 5.4. Polymerization of the small units derived either from the degradation products of lignin or from metabolites produced by microorganisms will form fulvic acids. However, by the way the diagram is constructed, it can be construed that fulvic acids are functioning only as intermediates in the diagenetic process. Diagenetic changes are processes toward geologic changes that perhaps pertain only to the change of humic acids into coal. Questions can also be raised what the rationale is in placing microbial metabolites next to coal or underneath fulvic acids.

Another possibility or hypothesis inserted here for further contemplation is the formation of humic compounds through trans-amination reactions of lignin monomers. Lignin monomers were indicated earlier to be formed from carbohydrates by the shikimic acid pathway. Decarboxylation of the end product, tyrosine (see Figure 2.6), may form a substance with the structure of an ammoniated coumaryl alcohol, or an ammoniated lignin monomer. Such a compound falls into the category of a ligno-protein compound, and hence can be considered as a prototype of a humic substance (Figure 5.5). Again a COOH and an OH group should be added to the phenyl core before the substance qualifies to be called a humic molecule. The ammonia, NH₃, has been derived from the decomposition of amino acids that can occur in both aerobic and anaerobic conditions, as illustrated previously by the deamination reactions 2.5 through 2.7.

5.3.2 Phenol-Protein Theory

This theory is called the *polyphenol theory* by Stevenson (1994) or the *phenol autoxidation theory* by Ziechmann (1994). However, in view of current concepts considering the humic acid core to be composed generally of an aromatic and nitrogenous substance, usually in the form of protein or amino acid, the present author proposes using the name *phenol-protein theory* in analogy to the ligno-protein theory. All these names can also be considered misnomers since not only phenols but also quinones are involved in the reactions forming humic matter. Quinones are phenol derivatives and often

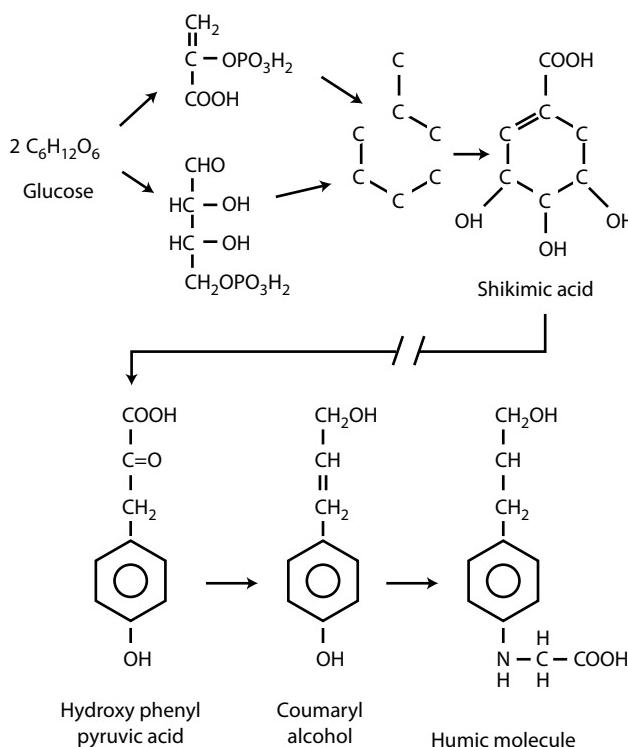


Figure 5.5 Abbreviated version of the synthesis of a ligno–protein complex through formation of lignin monomers according to the shikimic acid pathway. (Adapted from Schubert, W. J. *Lignin Biochemistry*. Academic Press, New York, 1965; Haider, K., J. P. Martin, Z. Filip, and E. Fustec-Mathon. Contribution of soil microbes to the formation of humic compounds. In: *Humic Substances. Their Structure and Function in the Biosphere*, D. Povolo, and H. L. Goleman (eds.). Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Center for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 71–85, 1975.)

play a dominant role in the formation of humic matter. Parts of the phenol–protein theory also tend to overlap with the ligno–protein theory. Many of the precursors and their reactions in humic matter formation are closely related and make one wonder about the necessity for advancing two different theories in the synthesis of humic matter. This was perhaps the idea behind Stevenson stating that the lignin concept has in fact been replaced by the polyphenol concept. Many of the theories on humification started indeed with lignin; however, it was the weathered products of lignin that were involved in producing the humic substances. Even in the ligno–protein hypothesis proposed by Flaig (1975), it is in fact the quinones and phenols resulting from the degradation of lignin that are used in creating the theory. Perhaps only

the sugar–amine condensation theory needs to be distinguished from the two above since it provides an explanation for the formation of humic matter in environments in which lignin and its derivatives are absent. However, since phenols and quinones are not derived only from the enzymatic degradation of lignin but can also be formed by microorganisms from sugars through the shikimic acid and acetate–malonate pathways, the feeling is that there is a need to also recognize the phenol theory as a distinct humification process. Many versions of the phenol, polyphenol, or quinone theories are available and some will be provided as illustrations in the subsections below.

In the phenol–protein theory, phenols, quinones, and amino acids are the key components for the formation of the humic molecule core. A simple reaction between gallic acid and a small amino acid molecule is shown in Figure 5.6. For comparison, a reaction is also provided between a hydroxyquinone and an amino acid. The formation of humic matter with the constituents as shown in Figure 5.6 can be used as a model for the abiotic condensation theory since the phenol and other reacting molecules are normally small in sizes. As stated earlier, the abiotic prescript applies only to the chemical reactions. The phenols and other participating components have been formed by biotic enzymatic reactions. This condensation theory assumes then that polymerization of the reaction products above is expected to yield first fulvic acid, which upon further condensation produces humic acid, and perhaps ultimately humin and kerogen. However, large polymers

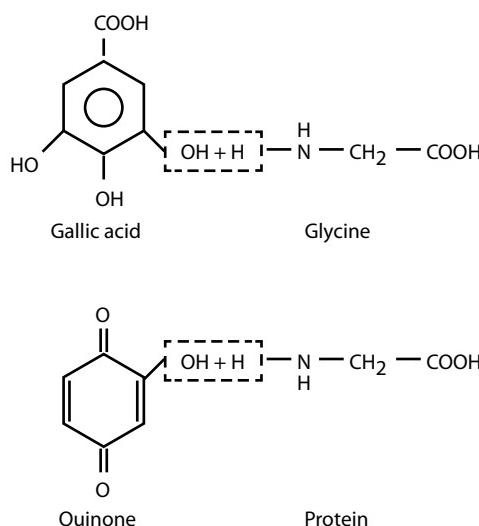


Figure 5.6 Possible reactions for humification according to the phenol–protein theory, involving (top) phenols, and (bottom) quinone and a simple amino acid molecule.

of phenols, quinones, and amino acids (protein) can also be formed before the formation of humic matter. When these large biopolymers enter into reactions, large humic molecules are expected to be formed first, such as humin, which upon degradation can be converted into humic acid, which in turn yields fulvic acid. This is a model example of a biopolymer degradation concept showing a principal difference from the original proposed by Hedges (1988). In the reactions as illustrated in Figure 5.6, the phenol biopolymers are not degradation products of lignin but have been synthesized by polymerization of the single phenol molecules. As indicated earlier, many different versions of the so-called phenol or polyphenol concept have been reported in the literature. For comparison with the author's ideas above, several of the major concepts are discussed in more detail below.

5.3.2.1 Hypothesis of Flaig (1975)

Flaig and coworkers (1975) suggested lignin to be the source or starting point in the formation of humic substances, which became well known in the literature as a classic example of the ligno-protein theory. However, after thorough examination, it is, in the author's opinion, a very controversial issue because it is phenol and/or quinone that is in fact the reaction component, whereas lignin is just a source for the production of quinones

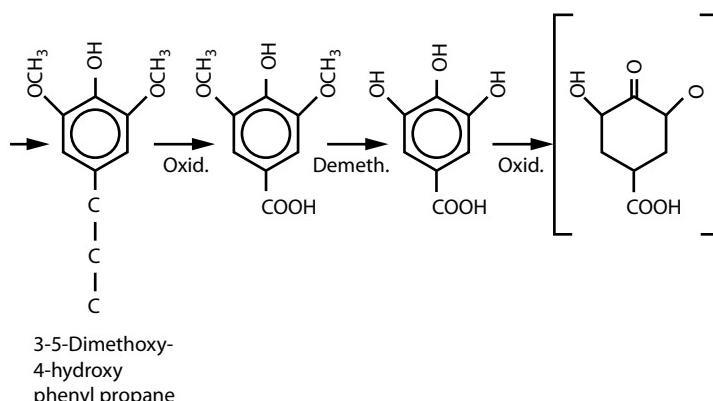


Figure 5.7 Degradation of a hardwood lignin monomer yielding quinone. (Adapted from Flaig, W. An introductory review on humic substances: Aspects of research on their genesis, their physical and chemical properties, and their effect on organisms. In: *Humic Substances. Their Structure and Function in the Biosphere*, D. Povoledo, and H. L. Golterman (eds.). Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Centre for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 19–42, 1975.)

as can be noticed from Figure 5.7. Lignin (3,5-dimethoxy-4-hydroxy phenyl propane) is assumed to be broken down by degradation reactions, oxidized into phenol units, followed by demethylation and further oxidation into quinones. Condensation of the quinone groups with amino acids and/or polysaccharides then yields humic acid-like substances. Because of the above, it is perhaps better to group Flaig's concept into the phenol–protein concept, in line with Stevenson's idea that the lignin theory has been replaced by the polyphenol concept. As stated before, in this case lignin just functions as a biopolymer source for the production of phenol or quinone molecules.

5.3.2.2 Phenol Hypothesis of Kononova (1961)

Kononova is of the opinion that the basic structural units of humic compounds are phenolic or quinoid substances, bonded to nitrogen-containing compounds and carbohydrates, the latter chiefly polyuronides. The inclusion of nitrogen-containing compounds and carbohydrates as fundamental units of the humic molecule is at that time a matter of much controversy. Several investigators regarded the latter as accidental contaminants, trapped in the maze work of the humic structure (Burges and Latter, 1960; Schnitzer and Khan, 1972). However, others supported Kononova's theory for the necessary participation of nitrogen compounds and carbohydrates in the formation of humic acids (Flaig et al., 1975). Kononova (1961) suggests that the following reaction occurs for the inclusion of nitrogen in the humic molecule (Figure 5.8). In her opinion, such a combination produces a stable condensation product of phenols and amino acids, which increases the stability of nitrogen in acid hydrolysis and microbial attack. The resulting product is, in general, more of a multicomponent system, called humic acid or fulvic acid. In Kononova's opinion, fulvic acid can be both the predecessor and the decomposition product of humic acid. The two humic compounds exhibit

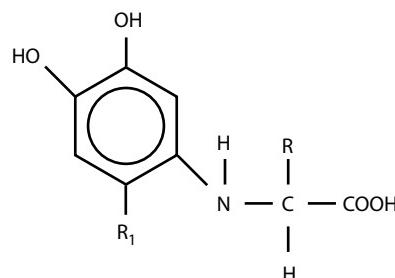


Figure 5.8 Structural hypothesis of Kononova (1961).

similar structural patterns but may differ in details of structural and chemical composition. For example, fulvic acid has a less condensed aromatic nucleus and has more highly developed peripheral components than humic acid.

5.3.2.3 Benzene Carboxylic Hypothesis of Schnitzer and Khan (1972)

This hypothesis is based on information obtained from chemical degradation of mostly fulvic acids. Schnitzer and Khan (1972) are of the opinion that humic substances must be broken down into smaller units to study their formation and structural chemistry. Thus far, four basic types of degradation procedures have been reported by these authors:

1. Oxidation with alkaline permanganate, nitric acid, H_2O_2 , or $CuO-NaOH$ mixture. The degradation products were invariably benzene carboxylic acids.
2. Reduction with Na amalgam or with Zn dust. Fulvic acids are also broken down into benzene derivatives.
3. Hydrolysis with hot water, acids, or bases. Fulvic acid yielded benzene derivatives, such as hydroxybenzoic and vanillic acids.
4. Biological degradation. Fulvic acid is decomposed with the aid of microorganisms (*Penicillium* sp., *Aspergillus* sp., and *Trichoderma* sp.). The compounds broken down from fulvic acid were also identified as benzene derivatives.

On the basis of the predominant discovery of benzene derivatives (Figure 5.9), Schnitzer and Khan (1972) assume that fulvic acid is composed of phenolic and benzene carboxylic acids, joined together by hydrogen bonds to form a polymeric structure. The latter contains many voids or openings in which other organic compounds can be trapped, such as amino acids and carbohydrates.

5.3.2.4 Phenol Dimer Hypothesis of Stevenson (1994)

Stevenson (1994) is of the opinion that humic substances can be formed by all the mechanisms discussed in the previous sections. The ligno–protein theory, polyphenol theory, and benzene carboxylic and sugar–amine condensation theory all contribute to building the complex structure of a humic molecule. A large number of precursor molecules take part in the condensation reaction,

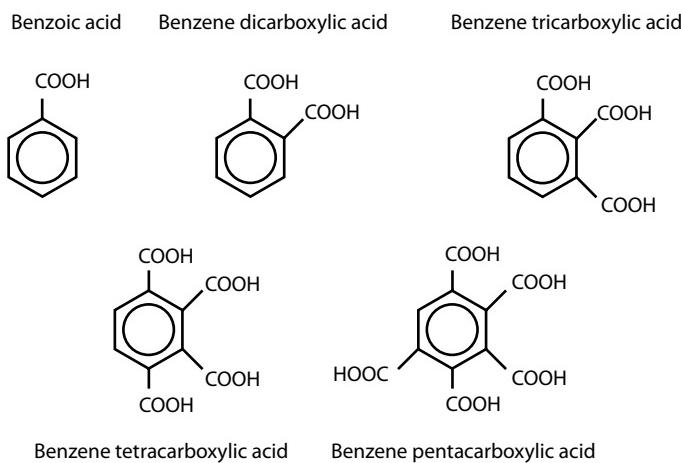


Figure 5.9 Benzene carboxylic derivatives produced by degradation procedures of Schnitzer and Khan (1972).

which can result in a variety of combinations. To simplify this extremely complex structural issue, Stevenson proposes the *dimer concept*. According to this concept, the core of a humic molecule consists of four building blocks: (1) a dimer, formed by coupling of two lignin monomers; (2) a phenol–amino acid complex; (3) a hydroxyquinone; and (4) a C₆–C₃ unit of lignin. The four building blocks have the roles of cards from a deck of structural units, and their combination leads to a great variety of core structures of humic matter. An example is shown in Figure 5.10.

As can be noticed in Figure 5.10, Stevenson has added an amino acid into his humic structure, suggesting that the core structure should also contain a nitrogen compound. As indicated above by the present author, a humic

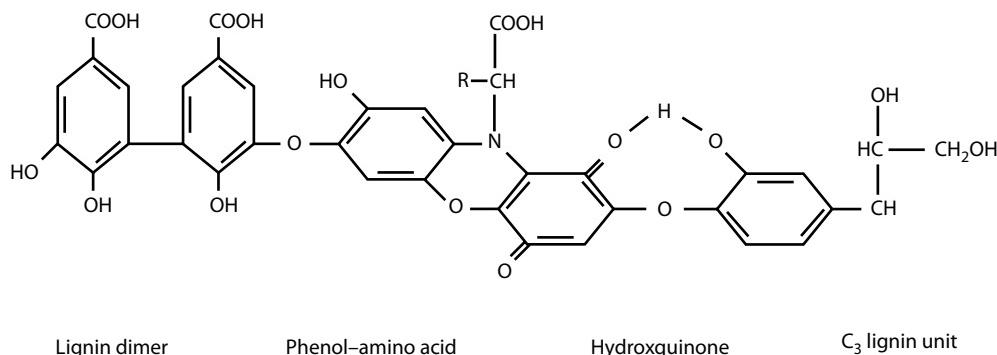


Figure 5.10 Phenol dimer hypothesis of Stevenson (1994).

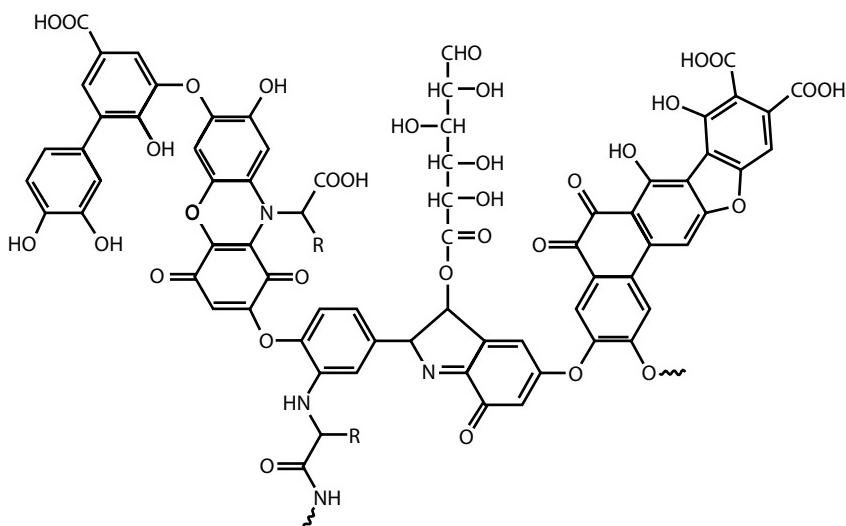


Figure 5.11 Humic acid structure by Yikrazuul. Note the nitrogenous (NH) group at the bottom crest and a carbohydrate tail stretching upward in the middle. (From http://commons.wikimedia.org/wiki/File:Humic_acid.svg, accessed February 7, 2013.)

core structure should correctly be composed of both phenol (or lignin) and a nitrogenous substance to qualify it as a humic acid substance. This is also the reason for the present author's suggestion to call the concept the phenol–protein concept, instead of the polyphenol or phenol concept. The version of a humic acid structure often claimed in many other books and printed announcements to be Stevenson's brand of humic acid structure does not show specifically the dimer concept as illustrated in Figure 5.10. The copies used in the other printed articles look more like a generalized humic acid structure composed mainly of phenol substances. It is an artistic piece of work created by Yikrazuul, claiming to release it into the public domain for use by others. For comparison, this version of a humic acid structure is provided in Figure 5.11. It is interesting to note that this humic acid structure also includes an NH and a carbohydrate group, making it comply scientifically with the concept of humic acid as a phenol–protein association.

5.3.3 Micellar Hypothesis of Wershaw (1986)

The concept was introduced by Wershaw in 1986 and resubmitted in 1999. It is examined further by Tan (2011a, b) who called the process of micelle formation *micellization*, and who also showed that the micelles formed can

assume different sizes and shapes, from single spheres, rectangular bilayers called membranes, to cylinders. As proposed originally by Wershaw (1986), decomposition of plant residue provided a great amount of small broken fragments at the macromolecular and micromolecular levels. Some of them are in the form of amphiphiles, which in solution tend to aggregate into micelles. These micelles are Wershaw's perception of humic substances. It was Tan (2011a, b) who has advanced the model at nanolevels because of his belief that decomposition will also produce large amounts of nanosized fragments. The micellar model of humic matter has been discussed in detail in Chapter 3, Section 3.5, and is here listed for completeness and for underscoring it also as one of the major advances in humic acid chemistry at the turn of the century. By its introduction, the present author believes that Wershaw has unwittingly applied the principles of self-assemblages of humic acids on a macro scale long before Piccolo reported the idea of supramolecular chemistry in 2001.

5.3.4 Supramolecular Concept of Piccolo (2001)

The failure of preexisting concepts to provide a commonly acceptable molecular weight characteristic for humic substances was allegedly one reason for Piccolo (2001, 2002) to turn humic acid science from a biopolymer concept into a supramolecular concept. Piccolo and coworkers arrived at the idea, in fact, from results of their analysis of humic acids using low-pressure (LPSEC) and especially high-pressure size exclusion chromatography (HPSEC) with Sephadex. They claim that in their experiments, the amount of large humic molecules eluted first was decreased, whereas that of the smaller molecules eluted last had increased substantially owing to several pretreatments of the humic samples, e.g., adding carboxylic acids and lowering the pH of the samples. The above results were taken as indications of humic acids being random associations of small molecules held by weak bonds. No additional analyses have been conducted by Piccolo's group to justify and support the controversial results obtained by the use of Sephadex and pretreatments. For more details on Piccolo's supramolecular concept, the readers are referred to Chapter 3, Section 3.6.

5.3.5 Humo–Nanotube–Membrane Hypothesis

The nanotube membrane concept in the formation of humic substances was introduced in 2011 by the present author as a result of his discoveries

of nanotubes and nanotube membrane structures of humic acids in his scanning electron microscopic (SEM) investigations (Tan, 1985, 2011a, b). The principles of nanochemistry are now applied by the author to explain the formation of humic substances exhibiting such characteristic structural features. A detailed discussion on nanochemistry and the nanotube membrane concept is provided in Chapter 3, Section 3.7. The process of spontaneous self-assembly of nanoparticles forming humic associations is called *nanofabrication* by the present author. In contrast to Piccolo's (2002) concept of random assembling producing structureless assemblages of humic substances, nanofabrication yields humic assemblies with distinct forms of well-ordered identifiable structures. At the smallest end of the size scale, they may often appear as clusters, whereas at the other (coarser) end, the assemblies can take the shape of tubes, rods, micelles, spheres, and membranes, to name just a few. Hence, they are often named according to their characteristic shape and form, such as nanotubes, membrane nanotubes (*cyanomes*), nanotube membranes, nanofibers as in assemblies of peptide amphiphiles, and more. Nanotube membranes can take the form of open-ended single nanotubes or can be assembled like the cells of a beehive honeycomb (Figure 5.2). Whatever the shape or form, it takes at least an amphiphile and a carbohydrate (or lignin derivative) to form a core that qualifies to be called a humic acid core as illustrated in Figure 5.12. In the reaction at the top of the figure, the amphiphilic head is negatively charged because of its carboxyl content in COO^- form that can attach to $^+H-\text{O}-H^+$ (water) serving as a bridge to a carbohydrate (fructose). In the author's opinion, water bridging is the most common attractive force occurring in soil ecosystems. It is considered a weak force but strong enough to hold the assemblages together. Since water is present as one of the four major soil constituents, it is more than logical that by its mere presence it is readily available to contribute toward instantly connecting the above organic components together. Second in rank is perhaps cationic bridging since it takes time for the ions to form first. Water bridging also takes place similarly through the individual amphiphilic heads of micelles (middle reaction) and membranes (bottom reaction) connecting each of them with a lignin derivative. The latter two reactions are examples of a theoretical formation of humic associations with an aromatic–protein core characteristic of terrestrial origins. On the other hand, the reaction at the top of Figure 5.12 is representative for formation of aquatic humic substances, exhibiting more carbohydrate–protein cores.

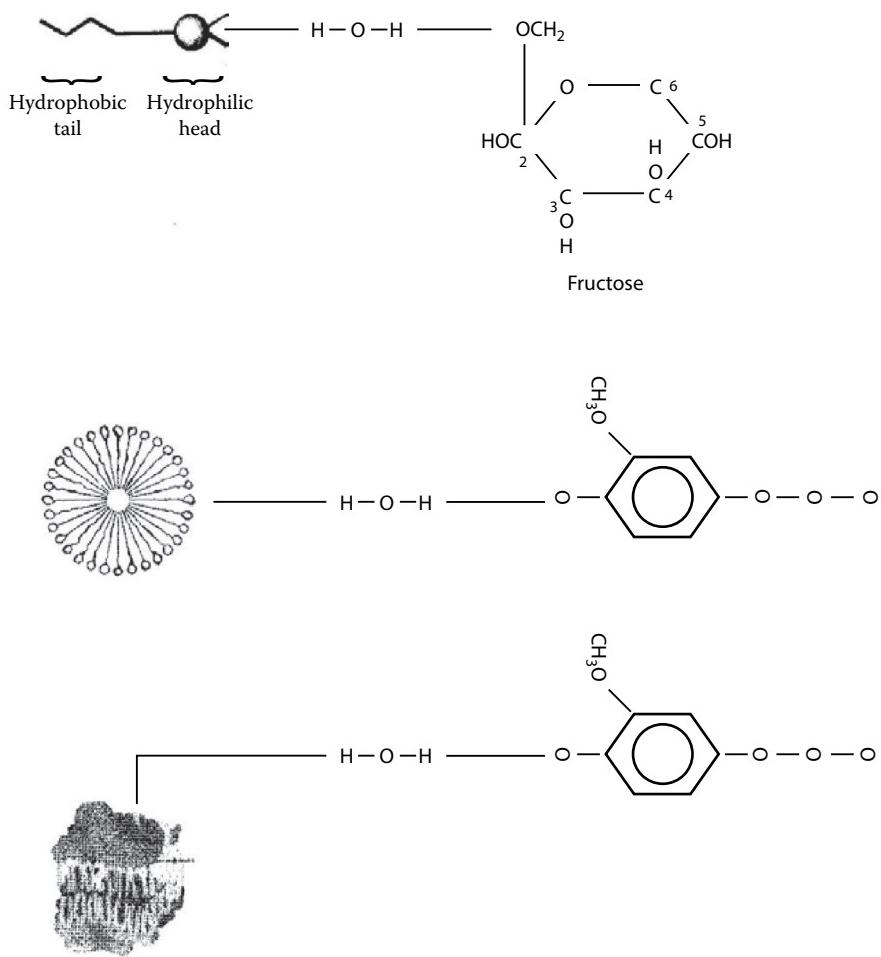


Figure 5.12 Formation of self-assemblages of humic acid cores by water bridging between (top) an amphiphile and a carbohydrate. The hydrophilic head contains carboxyl groups in the form of COO^- ; (middle) a micelle and a lignin derivative; and (bottom) a membrane and a lignin derivative. (Adapted from <http://www.assistanceescolaire.com> and <http://www.accessscience.com>, accessed March 11, 2013.)

5.3.6 Sugar–Amine Condensation Theory

This theory is also reported under different names and, as discussed earlier, has been called the melanoidin pathway by some (Hatcher et al., 1985; Nissenbaum and Kaplan, 1972) and the Maillard reaction by others (Ziechmann, 1994; Stevenson, 1994). It is a hypothesis using sugar and

amine as starting and key components for the synthesis of humic matter, and no lignin derivatives are required. The reaction, reported for the first time by Maillard in 1911, can take place nonenzymatically or abiotically between glucose and amine, producing melanins as end products. Ziechmann (1994) reports that glycine can substitute for the amine, which in the first step of the reaction with glucose yields glucosylamine. The latter, also called glycosylamine by other authors (Stevenson, 1994), will eventually be converted into melanoidins, and subsequent polymerization or condensation of the melanoidins produces humic matter. Melanins or melanoidins are pigments that are used in the food industry for coloring and flavor because of the aroma they carry. They also occur in nature and are believed to exhibit chemical properties characteristic of humic substances, especially of humin and kerogen in marine sediments (Hatcher et al., 1985; Nissenbaum and Kaplan, 1972). The name melanin has been applied in U.S. soil taxonomy for the development of *melanic* (Greek *melas-anos* = black) *epipedons*, meaning surface soils, rich in humic acids, hence exhibiting intense black colors as found in andosols (Soil Survey Staff, 1990).

The Maillard reaction as reported by Stevenson (1994) involves a very complex series of chemical processes. Sugar, the starting point, is converted first into a Schiff base by reaction with an amine. The latter is then converted into an O-substituted glycosylamine, which subsequently undergoes complicated *Amadori rearrangement* processes. The rearrangement products are then subjected again to a series of additional reactions, which make the concept confusing and mind boggling. For those interested, reference is made to Stevenson (1994) and Ziechmann (1994) for details and specifics of this chain of reactions. However, for the purpose of increasing comprehension, a simple reaction is provided in Figure 5.13 to illustrate the interaction between glucose and an amine. For comparison, a reaction between glucose and glycine is also provided. The basic molecular structures shown in the figure, composed of carbohydrates and nitrogenous compounds, correspond closely to basic structures advanced for autochthonous aquatic humic matter. As discussed earlier, plant materials in marine environments do not require lignin and are composed mostly of carbohydrates. Therefore, this theory indicates that marine humic matter, not affected by terrestrial material, is composed mainly of carbohydrate–protein complexes.

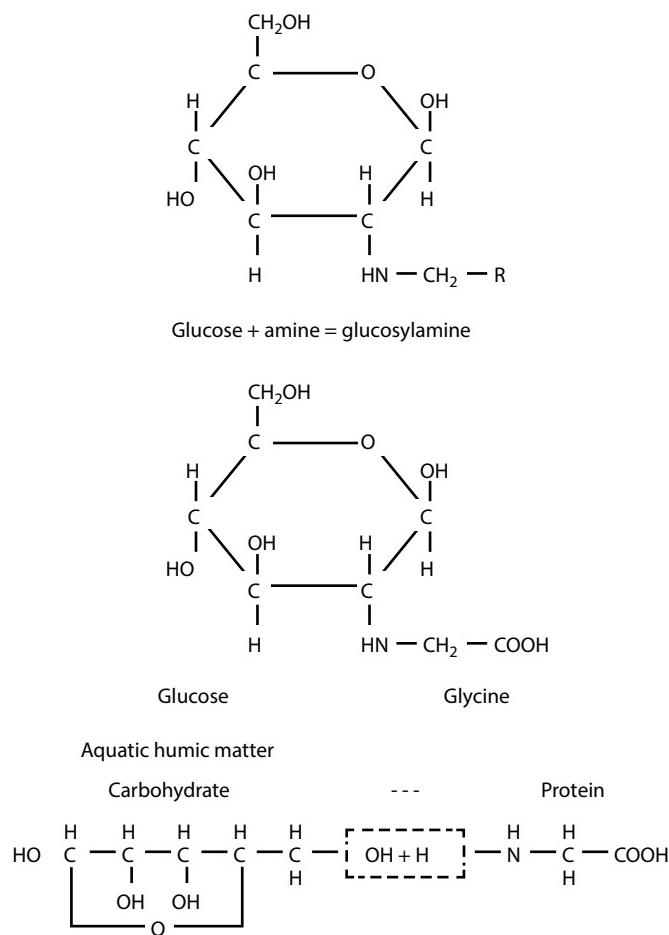


Figure 5.13 Simplified version of the reactions between (top) glucose and an amine, and (middle) glucose and glycine with the carbohydrate structures reflecting the ring or cyclic structures, and (bottom) a reaction between a simple protein and carbohydrate drawn in an open-chain structure.

5.3.6.1 Issue of Aromatic versus Aliphatic Nature of Humic Substances

As indicated by Susic (2008), the ligno-protein theory has dominated humic acid chemistry so much that humic substances are considered to be only aromatic in structure. The inclusion of nitrogen-containing compounds and carbohydrates in the core structure of the humic molecule has been remained for decades a matter of big controversy. Many prominent investigators disagree on the aliphatic nature of humic substances. Including especially carbohydrates in the humic structure is unthinkable (Burges and Latter, 1960;

Schnitzer and Khan, 1972), let alone agreeing on the idea that carbohydrates can create humic compounds. They are of the opinion that carbohydrates are contaminants that can or must be removed by thorough purification methods. However, a number of investigations point to the presence of polysaccharides as an integral part of a humic molecule (Tan and Clark, 1968; Clark and Tan, 1969; Tan, 1975). As illustrated in Figure 5.14, Tan and coworkers have discovered hymatmelanic acid to contain polysaccharide constituents in its core structure. Regretfully, this discovery seems to be ignored, but this idea seems to have increased in importance with the development of the concept of aquatic organic matter as spearheaded by geologists, geochemists, and hydrologists of the U.S. Geological Survey, Denver, Colorado. The advent of the aquatic concept of organic matter and its humic components at the start of the millennium, as discussed in detail in the preceding sections and especially in Chapters 1 and 4, has no doubt awokened many humic scientists and convinced them on the importance of carbohydrates in the synthesis of humic matter. The concepts of aquatic biomass and aquatic organic matter have been examined in detail in Chapter 1 (Sections 1.2.4 and 1.3.3). Surrounded completely by water during their entire growth, it appears that true indigenous aquatic plants do not need

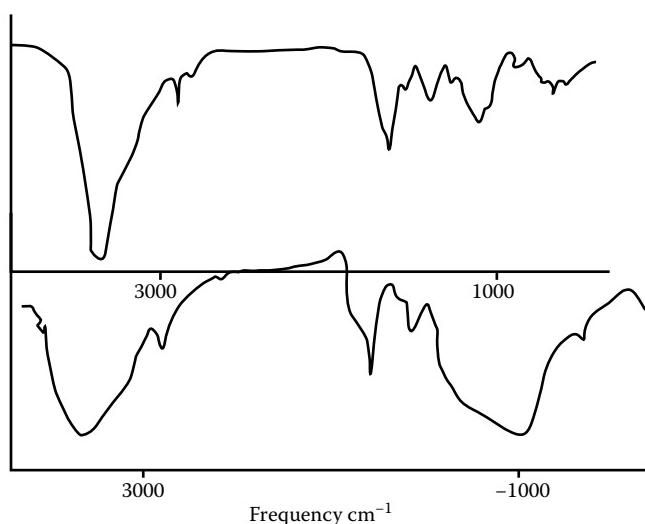


Figure 5.14 Infrared absorption spectra of (top) polysaccharide from *Spirulina* sp. and (bottom) polysaccharide from hymatmelanic acid extracted from soil. (From Fang, Y. et al., *Int. J. Nanomed.*, 7, 835–844, 2012; Tan, K. H., and F. E. Clark, *Geoderma*, 2, 245–255, 1968.)

lignin in their system. The composition of autochthonous organic matter is therefore more likely less lignoid and phenolic in character than allochthonous organic matter. They have been formed from indigenous aquatic organisms that exhibit low lignin contents, hence low aromatic structures, and they are instead substantially higher in carbohydrates, therefore called more aliphatic in structure. This is especially true for aquatic organisms such as algae, phytoplankton, kelp, and krill. It is noted that in big lakes and oceans most of the humic matter is autochthonous and aliphatic in character. Only on the beaches, shores, or coastal zones and in lagoons or estuaries is there a mixture with allochthonous or terrestrial materials. In the oceans remote from any terrestrial influence, aquatic humic matter is assumed to be derived from dead phytoplankton and/or algae (Kalle, 1966; McKnight et al., 1991, 1994). As can be noticed from the data in Table 5.1, aquatic organisms, such as plankton and diatom, the sources for formation of aquatic organic matter, do not contain lignin at all as analyzed and reported by Martinelli (2009) and Hunt (1996). In contrast, pine needles and oak leaves contain 17% and 37% lignin, respectively. The composition as revealed in Table 5.1 is in strong support of aquatic humic substances being carbohydrate–protein associations, as suggested by the present author with his reactions shown in Figure 5.13. The issue above on the aliphatic nature of aquatic humic matter has been examined in some depth in Chapter 4, Section 4.2.3.

Table 5.1 Biopolymer Composition of Plankton, Diatom, Pine Needles, and Oak Leaves in Weight Percentages

| | <i>Carbohydrate</i> | <i>Protein</i> | <i>Lignin</i> | <i>Lipid</i> |
|---------------|---------------------|----------------|---------------|--------------|
| Phytoplankton | 66 | 23 | 0 | 11 |
| Zooplankton | 22 | 60 | 0 | 18 |
| Diatoms | 63 | 29 | 0 | 8 |
| Pine needles | 47 | 8 | 17 | 28 |
| Oak leaves | 52 | 6 | 37 | 5 |

Source: Martinelli, G. Petroleum chemistry, Chapter 4. In: *Petroleum Engineering Upstream*, E. Mesini, and P. Macini (eds.). UNESCO, EOLSS Publishers, Oxford, UK. <http://www.eolss.net>, 2009; Hunt, J. M. 1996. *Petroleum. Geochemistry and Geology*, Second Edition. W. H. Freeman, New York; Orem, W. H., and P. G. Hatcher, *Int. J. Coal. Geol.*, 8, 33–54, 1989.

5.4 Statistical Modeling of Humification

In today's computer age, statistical modeling of almost any process in nature is becoming a great fashion. Statistical formulas abound in soil chemistry and other sciences, and many are so complicated that the message they want to convey is often obscured by the maze of symbols and pages of computations. Although not much is known yet on these efforts in humic acid chemistry, the limited information available shows a similar tendency in statistical modeling of the humification process. Some are reasonably easy to follow, whereas others are difficult to fathom their rationale and applicability. To make more sense of the complicated statistics, the following attempt is made to present and explain in simpler terms some of the theories available on statistical modeling of the humification process.

5.4.1 Humification Indexes

Attempts have been made to express the degree of humification in terms of optical density values, extinction, or absorbance. It is a controversial issue because some U.S. scientists disagree, whereas others—in particular scientists of German schools—are in strong support of it. It is, otherwise, a very easy and simple method. By plotting the logarithm of the absorbance against the wavelengths in the visible light range, an absorption spectrum of humic or fulvic acid is usually produced in the form of a straight line (Figure 5.15). The slope of such a line or curve is taken as a characteristic for differentiating the humic substances. The lighter the color of the humic substance, the steeper the slope, whereas the darker the color the more horizontal the spectral line tends to be. Fulvic acids are usually characterized by spectra with the steepest slopes, compared with humic acids (Tan, 2000; Tan and Van Schuylenborgh, 1961; Kumada, 1955, 1987). Hence, the degree of inclination of the spectral curves may translate into the rate or degree of the humification process. This inclination or slope of the spectral curve can be expressed statistically in several ways. It can be formulated in terms of a ratio or quotient of the absorbance at two arbitrary selected wavelengths, e.g., absorbance or extinction at 400 and 600 nm, called the color ratio E_4/E_6 or $Q_{4/6}$ (Springer, 1934; Tan and Van Schuylenborgh, 1961; Flaig et al., 1975; Kononova, 1966):

$$E_4/E_6 \text{ or } Q_{4/6} = \frac{\text{Log absorbance at } 400 \text{ nm}}{\text{Log absorbance at } 600 \text{ nm}} \quad (5.1)$$

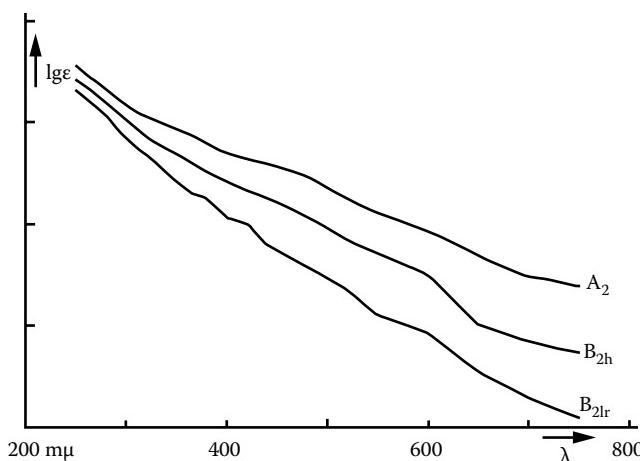


Figure 5.15 Absorption spectra of humic substances extracted from a tropical spodosol (humic–iron podzol) in Indonesia exhibiting differences in slope. Humic matter from the A_2 horizon has the weakest slope compared with that contained in the B_{2lr} horizon. (See also Tan, K. H., and J. Van Schuylenborgh, *Neth. J. Agric. Sci.*, 9, 174–180, 1961; Tan, K. H., and J. E. Giddens, *Geoderma*, 8, 221–229, 1972.)

A high color ratio, 7–8 or higher, corresponds to steep curves, usually observed for fulvic acids. On the other hand, a low color ratio, 3–5, indicates curves that are less steep, normally exhibited by humic acids. Consequently, the smaller the value of the color ratio, the greater will be the degree of humification (Tan and Giddens, 1972). This conclusion seems to be in close agreement with the contention of Chen et al. (1977), indicating the presence of an inverse relationship between E_4/E_6 ratios and degree of condensation of humic matter. An inverse relationship has also been cited by Stevenson (1994) between the color ratio and the mean residence time of humic matter. Humic substances exhibiting the highest E_4/E_6 ratios are the lowest in mean residence time. Consequently, fulvic acids tend to possess a shorter lifetime in soils than humic acids.

The slope of the spectral curve can also be expressed as the difference between the log values of the respective extinctions, and its value can also be used as a humification index (Tan and Van Schuylenborgh, 1961; Kumada, 1987):

$$\Delta \log E = \log E_{400} - \log E_{600} \quad (5.2)$$

where E = extinction or absorbance. The values for $\Delta \log E$ may vary from 1.1 or higher for less humified material, e.g., stable manure, to 0.6 or smaller for humic acids in andosols.

5.4.2 Stability Coefficient of Humus

The absorbance or extinction of humic solutions has also been applied by Hargitai (1955) for the formulation of a stability coefficient of humus as follows:

$$K = \frac{E_{\text{NaF}}}{E_{\text{NaOH}} H_c} \quad (5.3)$$

in which K = the stability coefficient of humus, E_{NaF} = extinction coefficient of humic acid extract in NaF, E_{NaOH} = extinction coefficient of humic acid solution in 0.5% NaOH, and H_c = humus content in percent.

Hargitai (1997) assumes that the K value relates the rate of solubility of humic acid in NaOH and the extent of mobilization in NaF of long-chain humified components. However, in the opinion of the present author, this is not what Equation 5.3 wants to convey. The use of the ratio $E_{\text{NaF}}/E_{\text{NaOH}}H_c$ refers to the amount of humic acid soluble in NaF in relation to that dissolved in NaOH times the humus concentration; in other words, the amount of humic acid soluble in NaF per the amount of NaOH soluble humic acid and humus concentration. Consequently, the ratio indicates that the higher the value of E_{NaF} , the larger will be the amounts of humic acid dissolved by NaF, hence the smaller will be the values for the denominators E_{NaOH} and H_c . This translates into high K values, indicating that the humus is unstable. The latter is perhaps being used or “destroyed” by humification. The relationship between high K values and humification seems to be supported by the author’s experiments on C/N ratios, where an increase in K values is noted to be closely related to a decrease in C/N ratios. Nonhumified organic residue usually exhibits a high C/N ratio (= 80 for straw), and humic acids are characterized by low C/N ratios (10–15). Therefore, the increase in K value may well mean an increased rate of humification.

5.4.3 Humification Model

A statistical model on humification is presented by Ziechmann (1994) and Kappler and Ziechmann (1969). Although at first glance, the model looks reasonably simple, it turns out to be a complicated model. It is apparently developed on the basis that a system of humic substances can be separated into several fractions: F_1 , F_2 , F_3 , to F_n . The molecular weight values increase from F_1 to F_n , implying that humification increases in the same direction. The authors above claim that the humification process, H , of such a system

can be defined as the negative sum of the products of the possible transition fraction and its logarithm. In their opinion, this definition assumes a Markhoff model, which can be written as follows:

$$H = - \sum p_i \cdot \log p_i \quad (5.4)$$

in which p_i = the relative mass of fraction i , $p_i \geq 0$, and $\sum p_i = 1$. Such a formulation for humification appears to raise a number of questions. In many other books, authors often consider that the reader should know basic statistics, hence offer only sparse explanations of symbols and declarations of rules or assignments of limits, and the like. They perhaps forget to realize that there are a variety of readers examining the book who may not necessarily have the same expertise. In this case, the use of a negative sign in Equation 5.4 has to be justified, and assigning the sum of p_i to equal 1 explained properly. It is difficult to express the degree or rate of humification by negative values. Second, the definition does not correspond to or is in conflict with the assignment " $\sum p_i = 1$ " and its application. As will be explained below, calculation of an application provides strong evidence that the humification model should perhaps be written more properly in the following form:

$$H = - \left(\sum p_i \right) \log p_i \quad (5.5)$$

When the masses of humic substances are distributed uniformly over all the fractions, the authors state that a maximum has been reached in the humification process, and $p_i = 1/n$. The H function is accordingly solved by the authors as follows:

$$H = - \sum \frac{1}{n} \log \frac{1}{n} = - \log \frac{1}{n} = \log n \quad (5.6)$$

The answer, $\log n$, can only be obtained by using the formula as written in Equation 5.6, and in addition when $\sum 1/n = 1$, since, by definition, $\sum p_i = 1$. Another issue is the selection of the value $1/n$ at a maximum rate of humification. In view of the sum of p_i being defined by the authors to equal 1, whereas n is the number of fractions, the value $1/n$ would more reasonably refer to the *average* or *mean value* of H , instead of to the maximum value.

Chapter 6

Extraction and Fractionation of Humic Substances

6.1 Search for Extractants

A proper analytical procedure is necessary for achieving the purpose of extracting the real humic substances. Contrary to the general opinion, isolation and purification are considered here as integral parts of the extraction procedure. The aim is to obtain the real humic compounds isolated from each other and free from coextracted materials or contaminants. A mixture of humic acid, fulvic acid, protein, and other compounds cannot be called humic acid, before humic acid is separated from fulvic acid and cleaned by a purification procedure. In the chemical industry, a mixture of formic acid and other organic acids is also not called formic acid unless this acid is separated from the other acids and adequately purified.

The success of the extraction procedure depends on the use of the most correct extraction reagent. The early methods used NaOH, which was accepted as the ideal extractant for humic substances in the old days. However, with the advancement of humic acid chemistry in the twentieth century, its suitability was challenged by a number of scientists (Chaminade, 1946; Bremner, 1950; Dubach et al., 1963; Flaig et al., 1975). Concerns about creating artifacts by NaOH extraction started a worldwide search for new, perhaps more suitable reagents for the extraction of humic substances. Hayes (1985) has provided a detailed account of the theory and chemical characteristics that a proper extracting reagent should have for an effective extraction of humic substances, and quoted four chemical

properties for a reagent to be accepted as the ideal solvent. He considers water to also be an excellent reagent for extraction of humic matter. In contrast, a different concept for setting criteria was used by Stevenson (1994), who believes that an ideal extraction procedure should meet the following:

1. The method should lead to the isolation of unaltered material.
2. The extracted humic materials must be free of inorganic contaminants, such as clay and polyvalent cations.
3. Extraction should be complete, or nearly so, thereby ensuring representation of fractions from the entire molecular weight range.
4. The method should be universally applicable to all soils.

Criterion 2 is perhaps unrealistic and impractical. By using a soil sample, it is unavoidable to coextract inorganic and organic contaminants. However, this is a matter of purification and not an issue of extraction. The remaining three criteria have, in essence, been covered earlier and have been circulating for quite some time in a shorter version as presented below (Flaig et al., 1975; Bremner, 1954):

1. The reagent should have no effect on changing the physical and chemical nature of the humic substances extracted.
2. The reagent should be able to quantitatively remove or isolate the humic substances from soils.

Over the years, a number of inorganic solvents have been tested (Schnitzer and Khan, 1972). In addition, several complexing agents and a variety of organic solvents have also been examined. In Hayes' (1985) opinion, these reagents must be capable of dealing with the polyelectrolyte behavior of humic substances and overcoming secondary forces in the solvent and macromolecular systems, whatever they are, if a proper dissolution is desired. Several methods have since been developed and are currently available for the extraction and isolation of humic substances from soils. To name a few, analytical procedures have been proposed by the International Humic Acid Society (Hayes et al., 1997), Soil Science Society of America (SSSA) (Schnitzer, 1982b), Stevenson (1994), Tan (1996), and many more. Ignoring the variations present in the different methods for manipulation of the analyses, the key ingredient in all the above-mentioned methods is still NaOH; hence, the uncertainty facing investigators of extracting real

humic compounds from soils is still alive. However, some consolation can be found from several studies indicating the methods using NaOH to be reliable within certain limits and suitable for the intended purpose (Tan et al., 1994). This observation is supported by Orlov (1985), who, as indicated before, is a firm believer that NaOH extraction yields humic products that are reproducible from the quantitative as well as from the qualitative aspects. While all of the above applies only to extraction of humic matter from soils, peat, and other deposits, extraction and isolation of aquatic humic matter require a somewhat different approach. The humic substances are already present in solution, or at least in fine suspension form. However, because of their low concentrations in the aqueous medium, a preparative method for concentrating the humic matter is necessary before sufficient amounts can be isolated for analytical and other purposes. Several methods are available to meet this objective, and one of the most modern methods is the resin adsorption technique, which will be discussed separately in a section below.

6.1.1 Inorganic Reagents

Over the years, many inorganic solvents have in fact been evaluated for their effectiveness in extracting humic compounds (Stevenson, 1965; Schnitzer et al., 1959), usually with mixed results in meeting the two conditions for an ideal extractant as indicated above. Some of the reagents, e.g., dilute bases, can meet the condition set for the quantitative removal of humic fractions. However, all of these bases are suspected to have some effect on modifying the physical and chemical properties of the extracted humic substances (Flaig et al., 1975). The possibility of creating artifacts is still confronting investigators today. Some of the inorganic reagents used in extraction are listed in Table 6.1. Among the reagents listed, especially NaOH is believed to approach the two points above for an ideal extraction reagent more closely than the other listed chemicals. The use of NaOH can be traced back to the first attempts to extract humic matter by Achard, Doeberleiner, Berzelius, Mulder, and other pioneers in humic matter science (see Chapters 1 and 2). Since Oden's rediscovery of this reagent in 1919, the method of extraction with NaOH seems to have been the most widely accepted procedure for some time.

Today, the basics remain the same, but some modifications and refinements to Oden's original extraction procedure have been instituted corresponding to new modern standards. NaOH is believed to be the most

Table 6.1 Inorganic Reagents Used for Extraction of Humic Substances

| <i>Acids</i> | <i>Bases and Salts</i> |
|----------------------------|---|
| 0.1 N HCl | 0.1 N NaOH |
| 0.025 N HF | 0.5 N NaOH |
| 1% H_3BO_3 | 0.1 M Na_2CO_3 |
| | 0.5 M Na_2CO_3 , pH 10.5 |
| | 0.2 M Na citrate, pH 7.0 |
| | 0.1 M NaF |
| | 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$, pH 7.0 |
| | 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$, pH 9–10 |
| | 0.2 M $\text{Na}_2\text{-EDTA}$ |
| | 0.1 M $\text{Na}_2\text{B}_4\text{O}_7$ |
| | Urea |

effective reagent in quantitatively isolating humic substances from soils. Its easy removal during the purification process is an additional benefit. However, the use of this reagent is suspected to induce auto-oxidation of humic substances, and humic acids extracted by NaOH are reported to differ in C, N, and O contents from those extracted by other reagents (Hayes, 1985). To alleviate this problem, it is usually recommended to conduct the extraction under an N_2 gas atmosphere (Hayes, 1985; Schnitzer, 1982b; Choudri and Stevenson, 1957). However, indications are presented from other studies that the differences in amounts extracted and the properties of humic substances, attributed to NaOH extraction under air and N_2 gas, are very small. No definite trend is noted for extraction under an N_2 gas atmosphere to be superior to the conventional method with plain air (Bremner, 1950; Tan et al., 1991, 1994). The problem lies perhaps not so much in the type of extracting reagent, but more in the standard for comparison of humic compounds. No accurate standard humic acid is available or, better, scientists cannot agree about a humic acid compound that accurately represents the real compound in nature. Efforts made by the International Humic Substances Society (IHSS) for standardization of extraction procedures are helpful; however, its method has not been thoroughly tested and still requires a whole lot of comparison with other methods.

In using the NaOH method, the use of a 0.1 M NaOH solution is suggested because of its milder nature for extraction than 0.5 M NaOH (Tan, 1996; Pierce and Felbeck, 1975), decreasing in this way the chances for harmful alterations. For qualitative investigations, a very weak NaOH solution, of 0.001 to 0.01 M, is even better than 0.1 M NaOH. By rule, it is known that the stronger the NaOH solution, the more will be extracted, but the greater will be the chances for chemical changes to occur in the extracted humic matter.

Sodium pyrophosphate, $\text{Na}_4\text{P}_2\text{O}_7$, although not as effective as NaOH, is used frequently for the extraction of humic matter from soils high in sesquioxide contents. Chelation by the phosphate of Al, Fe, and other metal sesquioxidic ions is believed to increase the solubility of the humic substances, hence enhances their extraction (Kononova, 1961). Other chelating agents, e.g., EDTA, have also been used for a similar purpose in the extraction of humic matter. To increase the effectiveness of pyrophosphate, a solution with a pH of 9 to 10 is recommended. Nevertheless, the amount extracted is usually considerably less than that obtained with NaOH, but Stevenson (1994) believes that the chances for alterations are less if $\text{Na}_4\text{P}_2\text{O}_7$ is used, especially at pH 7.0. Although reports to the contrary are present, the use of pyrophosphate often eliminates the need of decalcifying calcareous soils before extraction, a pretreatment required with the NaOH procedure. The disadvantage of the $\text{Na}_4\text{P}_2\text{O}_7$ method is that it is very difficult to purify the extracted humic matter. Phosphate is known to be chelated strongly by humic substances. Although in a few cases, it has been noticed that humic fractions, isolated with $\text{Na}_4\text{P}_2\text{O}_7$, yield infrared spectra with better resolutions (Tan, 1978), the infrared features are often not characteristic of humic compounds. A comparative study on the effectiveness of NaOH and $\text{Na}_4\text{P}_2\text{O}_7$ extraction of humic acids by Orioli and Curvetto (1980) provides additional information showing that the pyrophosphate method has apparently not extracted three high molecular weight fractions that were identified in the NaOH extracts.

Extraction with acids, e.g., HCl, as conducted by Schnitzer et al. (1959), technically yields only fulvic acids, since only these humic fractions are soluble in acidic solutions. By definition, humic acids are insoluble in acidic condition, and the humic acids identified by Schnitzer and coworkers above are most probably degradation products due to hydrolysis by the acidic reagent.

6.1.2 Organic Reagents

The selection for using organic reagents in the extraction and isolation of humic matter from soils originates from the desire to avoid producing

chemical changes in the extracted product. A variety of organic solvents have been tested, and some are listed in Table 6.2. These substances have been used alone, as single reagents, or as mixtures at varying concentrations. Thus far, none of them has been satisfactory (Schnitzer and Khan, 1972), and the use of organic solvents appears to create more problems than with the inorganic solvents. Not only have they proven to be weaker extractants, but in contrast with the inorganic reagents, e.g., NaOH, which is easy to remove, the organic reagents are more difficult to remove in the purification process. Their possible interaction with humic substances is assumed to make it more difficult to purify the extracted humic fractions. In addition, the chances are enhanced not only for producing chemical

Table 6.2 Organic Reagents Used for Extraction of Humic Substances

| <i>Acids</i> | <i>Non-Acids</i> | |
|--------------|--------------------------|-------------------------|
| Formic acid | Acetonitrile | Acetylacetone |
| Oxalic acid | Benzene | Chloroform |
| | Dichloromethane | Dimethylformamide (DMF) |
| | Dimethylsulfoxide (DMSO) | Dioxane |
| | Dodecylsulfate | Ethanol |
| | Ether | Ethylenediamine |
| | Formamide | Hexamethylenetetramine |
| | Methylisobutyl ketone | Phenol |
| | Pyridine | Tetrahydrofuran |

Source: Schnitzer, M., and S. U. Khan. *Humic Substances in the Environment*. Marcel Dekker, New York, 1972; Hayes, M. H. B. Extraction of humic substances from soil. *Humic Substances in Soil, Sediment, and Water. Geochemistry, Isolation, and Characterization*. 1985. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Ziechmann, W. *Humic Substances*. George August Universität Göttingen, Bibliographischer Institut, Wissenschaftsverlag, Mannheim, Germany, 1994; Stevenson, F. J. *Humus Chemistry. Genesis, Composition, Reactions*, Second Edition. 1994. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Hayes, M. H. B., and R. L. Malcolm. Considerations of compositions and aspects of the structures of humic substances. *Humic Substances and Chemical Contaminants*, C. E. Clapp, M. H. B. Hayes, N. Senesi, P. R. Bloom, and P. M. Jardine (eds.). Proc. Workshop and Symposium Int. Humic Substances Soc., Soil Sci. Soc. Am., and Am. Soc. Agronomy, Anaheim, CA, October 26–27, 1997. Soil Sci. Soc. Am. Inc., Madison, WI, pp. 33–39, 2001.

changes but also for creating artifacts, due to incorporation of the organic reagents in the molecular structure of the humic molecule. Indications that the organic reagents are extracting lower amounts of humic matter than the inorganic solvents have been presented by Hayes (1985), whose analysis shows ethylenediamine (EDA) to be a poor solvent, and only by repeated extractions with EDA was the combined yield of humic matter comparable to that extracted by a 0.5 M NaOH solution. The data of the author above with methylsulfoxide and dimethylformamide (DMF) even show considerably less humic substances extracted than that by EDA. Indications for the possibility of chemical changes in the extracted humic matter are provided by the higher nitrogen contents noticed in humic substances extracted by Hayes (1985) with EDA, a compound containing nitrogen. To this fact can be added reports claiming that humic acids isolated by 0.5 and 0.1 M hydroxymethyl amine have carboxyl contents substantially different from those extracted with NaOH (Orioli and Curette, 1980). On the other hand, Alberts and Filip (1994) show that the chelation capacity of humic acids extracted by hexane and methylene chloride solutions is not much different from that of humic acids isolated with a mixture of 0.1 N NaP₂O₄ and NaOH solutions. The latter finding indicates that further investigations are perhaps needed to bring more light on the issue of chemical changes in humic matter caused by organic solvents. Organic acids, similarly to the inorganic acids, are poor choices as extracting reagents because their acidic reactions are incompatible with the concept of humic acids. Nevertheless, a lot of work has been conducted on extraction of humic substances with formic acid. This reagent is reported to extract 50% of the humic matter content in soils only in the presence of LiF, LiBr, or HBF₄, added to the formic acid solution. The added chemicals are assumed to break the hydrogen- or metal-complex bonds, enhancing in this way the dissolution of the humic substances (Stevenson, 1989).

Organic chelating agents have also proven to be less suitable for extraction of soil humic matter. The organic reagent acetylacetone (Table 6.2) has been used for isolation of humic substances in the B_h horizons of spodosols. It is reported to function by chelation of Al and Fe, enhancing the dissolution and extraction of the humic substances from the spodosols. However, the reagent is believed to be ineffective for extraction of humic matter in other soils (Stevenson, 1994).

According to Hayes and Malcolm (2001), good organic solvents have an electrostatic factor >140 and a pK_{HB} >2, as exhibited by DMF and dimethyl sulfoxide (DMSO). The pK_{HB} is explained as the capacity of the solvent as an H-acceptor; hence, the organic reagents DMF and DMSO are Brønsted–Lowry

bases. The authors add that to be considered the best, the solvents must exhibit also δ_p (dispersion force), δ_H (force of H-bonding), and δ_b (force as proton acceptor) greater than 6.5 and 5, respectively. Apparently, the authors forgot a third value, since three kinds of δ_s were stated. They concluded that water satisfied all the criteria, but, unfortunately, it is a very poor solvent and extractant for humic substances. It is also puzzling why so much emphasis is placed on the parameters above, since at the end, it is concluded that the use of DMF and DMSO also present problems in extraction and isolation of humic substances.

6.1.3 Reagents for Collecting Aquatic Humic Substances

6.1.3.1 Methods and Reagents

As indicated earlier, a different approach must be taken in the extraction and isolation of aquatic humic matter because the humic substances are already in finely dispersed forms, or in solution as considered by some authors.

They are, in fact, in suspension as macromolecular colloids in the 0.45 μm or smaller size fraction (Gaffney et al., 1996), a size below the clay size limit of 2 μm . Because of their low concentrations in water, it is necessary to concentrate them first in order to be able to isolate them properly, and at the same time obtain sufficient amounts of samples for analysis and investigations. A great number of methods have been developed for this purpose, which perhaps can be distinguished into physical and chemical methods. Aiken (1985) has given a detailed account of the merits of several of these techniques.

The major physical methods for concentrating the finely dispersed humic substances are vacuum distillation, freeze-drying, freeze concentration, superspeed centrifugation, and ultrafiltration. Since they do not require the use of chemical reagents, the proponents of these methods believe that formation of physical and chemical changes in the humic substances is avoided or minimized.

Chemical methods, on the other hand, employ either chemical agents or adsorbents. The chemical reagents are used in the precipitation method by which the humic substances are precipitated either with metals or by adjusting the pH to 2.0. The methods using adsorbents apply the basics of gel chromatography and appear to have attracted the most attention. A variety of adsorbents have been tested, e.g., anion resins, polysaccharides, polystyrene, polyamides, aluminum oxides, cellulose, and agar. Cationic resins are unsuitable since aquatic humic matter is also negative in charge. The gel materials include cross-linked polymers of polysaccharides, polystyrene,

polyamides, and the like, distributed under names such as Biogel, Cellogel, Sephadex, and Sephadex. However, these resins do not only isolate and concentrate but also fractionate the humic substance, which in this case is not the purpose of the analysis. XAD Amberlite resins have been suggested by Thurman and Malcolm (1981) as the most suitable sorbents for the preparative isolation of “dissolved” = humic substances. In contrast to Sephadex, Sephadex, and the like, the XAD resins do not fractionate but function mainly in isolating and concentrating the humic substances from water.

6.1.3.2 XAD Resin Issues

The XAD resin technique is reported to be superior to the physical methods and capable of concentrating humic matter from solutions with concentrations as low as <50 µg/L DOC, such as in groundwater, by repeated cycles of adsorption and desorption. A variety of Amberlite XAD resins are available for this purpose, e.g., XAD-1 to XAD-12. They are nonionic copolymers exhibiting macropores and large amounts of surface area. The sorption capacity of the resins is assumed to be affected by solution pH, hydrophobic behavior, and the solute's solubility in water (Aiken, 1985, 1988), which Thurman et al. (1978) explain as follows. At low pH, the resin will adsorb humic substances since the latter are noncharged (neutral or protonated) in the acidic medium. In contrast, at high pH, the humic matter is negatively charged, and charged particles will not be adsorbed by the resin. Of the types in the XAD series, the resins in the form of acrylic esters (XAD-7 and XAD-8) are preferred to those composed of styrene–divinylbenzene (XAD-1, XAD-2, and XAD-4). Recovery by elution of adsorbed material is more efficient and rapid with the two acrylic esters, which are hydrophilic in nature, than with the three other resins, which are more hydrophobic in character. Aiken (1985) claims that due to the hydrophilic character, the acrylic ester resins, XAD-7 and XAD-8, are capable of adsorbing more water and becoming wet more easily. These are essentially factors favoring the development of water bridging, enhancing adsorption of noncharged humic matter by relatively weak bonding. On the other hand, the difficulties in elution from the styrene–divinylbenzene resins are explained by the authors as affected by strong bonding resulting from hydrophobic and chelation interactions between the resins and the humic material. Between XAD-7 and XAD-8, Aiken (1985) suggests the use of XAD-8 because of the serious problems obtained with XAD-7 and NaOH, known as “bleeding.” However, Chen et al. (1977) is of the opinion that XAD-12 is a better adsorbent than XAD-8,

especially for the isolation of humic acids. Its stronger hydrophilic property than that of XAD-8 is assumed to result in attraction of more water molecules, producing more bridges for the sorption of humic acids as indicated above.

6.1.3.3 Alternative Types of Resins

Although many believe that the XAD resins are the best choices, other resins are also available for the purpose of isolating and concentrating humic substances from dilute solutions. For example, anion exchange resins have the potential for use in sorption and isolating humic substances from aquatic environments. The negatively charged humic molecules will be adsorbed readily by the positively charged resins, and the recovery of the adsorbed material is expected to be achieved easily by elution with an NaOH or by a neutral salt solution. Aiken (1988) recommends Duolite A-7, which he assumes to have a high adsorption capacity for anionic organic solutes that can be eluted rapidly when the amount adsorbed is limited from one-third to one-half of the resin's loading capacity. However, today, a variety of more modern types of anion exchange resins are in fact available for use in a rapid and simple isolation of aquatic humic matter. For example, Dowex SAR, a styrene–DVB copolymer containing trimethyl benzyl ammonium active groups; Dowex SBR, with dimethyl benzyl ammonium active groups; and Ionac NA-38, one of the Ionac series, an alkyl quaternary ammonium polystyrene resin, are possible reagents for use with the same purpose as Duolite A-7. This list should be realized as a scientific statement only and is not an endorsement of a specific product at all. Unfortunately, most of them have not attracted too much research attention and are still awaiting testing for their suitability in sorption of dispersed humic substances in natural waters. To be effective in sorption of humic matter, Aiken (1988) quoted Abrams (1975) that the anion exchange reagent should exhibit a macroporous structure, contain weak-base functional groups, and have a hydrophilic matrix that is highly negatively charged at pH 10. However, the latter is perhaps an error and is subject to a lot of argument. As a rule, anion exchange resins carry positive charges that are attracting negatively charged ions, called anions, and this is the reason why they are called anion exchange resins. A resin carrying positive and negative charges is not an anion exchange resin but an amphoteric compound. The anion exchange resins listed above are all strong bases, meaning that at pH 10 they are positively charged.

In contrast with the XAD resins where nonionic (neutral) humic substances are adsorbed, with the anion exchange method, only the ionic

form or negatively charged humic substance will be adsorbed. The force for attraction is electrostatic attraction, which provides for a stronger bonding than the water bridge bond with the XAD resins. Nevertheless, it is not expected to result in difficulties for recovery by elution of the adsorbed material. The disadvantage is that other negatively charged organic compounds are coadsorbed, making purification of the eluted humic substances somewhat more complex and tedious.

6.2 Terrestrial Humic Matter

6.2.1 Extraction Methods

As mentioned earlier, the several methods available for extraction of humic substances from soils, peat, and geologic deposits are in essence not much different from the basic procedure using NaOH. They only vary in pretreatments of the sample, fractionation, and in purification of the extracted materials. Hence the basic procedure widely used by the majority of scientists will be outlined below, and the variation in pretreatments, fractionation, and purification according to the IHSS, SSSA, and other authors will be provided in separate sections.

6.2.1.1 Pretreatments

The IHSS recommends acidifying the sample before extraction (Hayes, 1985). The soil is equilibrated first with 0.1 M HCl for 2 to 3 h, after which it is washed thoroughly with water followed by adjusting the soil pH to 7.0 with NaOH. The SSSA follows a similar procedure (Schnitzer, 1982b), but (Tan, 1996; Tan et al., 1994) suggests using such a pretreatment only for calcareous soil samples. Removal of the high calcium content seems to enhance extraction of humic matter. On the other hand, acidification of acidic soils, e.g., andosols, ultisols, and oxisols, is unnecessary and may even prove to create problems in the extraction of humic substances.

6.2.1.2 Extraction Procedure

The basic procedure most people adhere to is to mix a weighed amount of soil with a 0.1 M solution of NaOH at a soil/solution ratio of 1:5 and shake it continuously overnight. A soil/solution ratio of 1:10 is used by the IHSS. In

general, the ratio to be used depends on the organic content of soils. The higher ratio is preferred for use with soil samples rich in organic matter; hence, a ratio of 1:10 is recommended for extraction of peat. In the IHSS and SSSA procedures, the mixture is placed and shaken in a nitrogen atmosphere. Choudri and Stevenson (1957) even suggest adding stannous chloride as an antioxidant to prevent auto-oxidation of the humic substances. However, Stevenson (1994) deleted the use of both the nitrogen atmosphere and SnCl_2 . This makes the extraction procedure faster and easier, and as indicated earlier it has no effect at all on the quantity and quality of the extracted product.

An example of the basic extraction procedure is outlined in Figure 6.1 and will be discussed in detail as follows: Ten grams of soil (sieved to pass a 2-mm sieve) is weighed in a propylene centrifuge tube. If an N_2 -atmosphere is desired, the soil is placed in a vacuum Erlenmeyer flask. Fifty milliliters of 0.1 M NaOH is added and N_2 gas is bubbled into the flask until all the air is replaced by nitrogen. The flask is stoppered airtight and the mixture is shaken continuously overnight. The dark-colored supernatant is separated from the soil the next day, by centrifugation at 10,000 rpm for 15 min. After decanting the supernatant in a separate flask, the soil residue is washed with 50 mL distilled water and the colored water is centrifuged, decanted, and

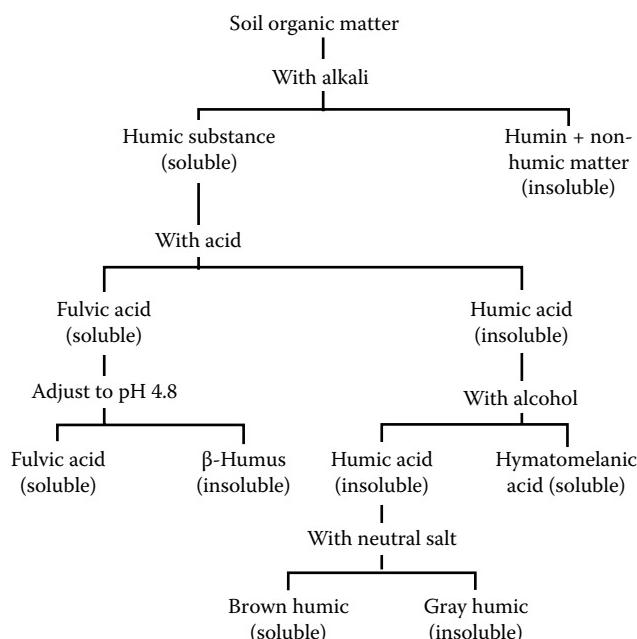


Figure 6.1 Flow sheet for extraction of humic substances from soils, peat, organic matter, and other terrestrial deposits.

combined with the previous extract. If desired for quantitative purposes, the extraction can be repeated once or several times by adding again 50 mL NaOH, etc. The combined supernatants, containing the humic substances, are centrifuged again at 15,000 rpm for 15 min to ensure complete removal of fine colloidal clays. The precipitate is discarded and the humic solution is collected, which should be as clear as a pair of dark sunglasses. It is then acidified to pH 2.0 by adding drops of HCl to precipitate the humic acid fraction. The supernatant, containing fulvic acid, is then separated from the precipitate (= humic acid) by centrifugation at 10,000 rpm for 5 to 10 min. Both the humic acid and the fulvic acid fractions are collected for further purification and fractionation, as discussed below. Obtaining humic and fulvic acids is considered here not as a fractioning process of a humic substance but as an extraction and isolation procedure necessary to obtain both substances present in the mixture.

6.2.1.3 Purification of Humic Acid

The crude humic acid (HA) precipitate obtained above is redissolved with 0.1 M NaOH and centrifuged at 10,000 rpm. The undissolved fraction is discarded, and the dissolved fraction is acidified again to pH 2.0. The precipitate is separated from the solution after acidification by centrifugation at 10,000 rpm for 5 min, and the supernatant is discarded. The HA precipitate at the bottom of the flask is shaken loose with 50 mL of a very dilute HCl + HF mixture in order to reduce the ash and silica content. After shaking, the mixture is centrifuged and the HCl + HF extract is discarded, but the HA precipitate is saved. This precipitate is then washed thoroughly with distilled water, centrifuged, and the wash water is discarded. For a final purification step, Tan (1996) suggests redissolving the HA precipitate with 50 mL of 0.1 M NaOH and allowing the solution to pass through an H-saturated cation exchange (Dowex 50-X8) column, a procedure adapted from Lakatos et al. (1977). The eluted HA solution has a pH between 2.0 and 3.0, because the humic acid is highly protonated. The HA remains in “solution,” although apparently as an unstable solution. Such a behavior of HA corresponds to that of aquatic HA, which stays in solution in the Okefenokee Swamp black water with a pH of 3.8 (Tan, 1993). This purified HA can be used as such for analysis, or it can be freeze-dried and stored in an amber-colored flask for later use.

An alternative purification procedure without the use of a cation exchanger is to transfer the humic solution after HCl + HF treatment into

dialysis tubings, and dialyze it against distilled water for three to four nights. Care must be taken to refresh the water every 6 to 12 h. This procedure is more time consuming but the results are the same as with the cation exchange method.

6.2.1.4 Purification of Fulvic Acid

The colored supernatant containing the fulvic acid (FA) fraction is purified by passing through an Amberlite XAD-8 column, as recommended by the IHSS. For the preparation of an XAD-8 column, see Section 6.4.1. Fulvic acid retained by the XAD resin is washed twice by elution with distilled water to remove carbohydrates and extraneous coadsorbed compounds. The washed FA is then eluted from the column with a 0.1 M NaOH solution, after which it is allowed to flow through an H-saturated cation exchange (Dowex 50-X8) column for a final purification. The purified FA can be used as such for analysis or it can be freeze-dried, weighed, and stored in an amber-colored flask for later use. This XAD-purified fulvic acid is called generic fulvic acid by Stevenson (1994).

6.3 Fractionation of Humic Substances

6.3.1 Fractionation of Humic Acid

As stated above, the procedure for obtaining humic acid and fulvic acid is in fact an isolation process, and purification is an integral part of the extraction method. Extraction and isolation are not fractionation procedures as many authors believe them to be. The following analogy is used to emphasize this contention. In a mixture of soil organic matter composed of clay, silt, sand, leaves, twigs, flowers, and roots, the extraction and isolation of the leaves and twigs from the soil mixture can hardly be called a fractionation process. The term fractionation can be used only when after isolation of the leaves from the twigs, the leaves are cut into leaf blades and stems. This applies also to the fractionation of humic and fulvic acids, as embodied in the definition presented by Swift (1985) stating that fractionation is the subdividing of humic substances according to some property. This is supported by a statement by Leenheer (1985) implying that fractionation procedures must be clearly distinguished from extraction and isolation procedures. Hence, what both Swift and Leenheer want to imply is that after the humic substances have been extracted and

humic acid properly isolated from fulvic acid, each of them can then be broken down into subfractions. Three reasons have been cited by Swift (1985) for the necessity of fractionation of humic substances: (1) to facilitate determination of the physical and chemical properties, (2) to determine the range of variation in properties, and (3) for use in characterization or in fingerprinting. These reasons can only be realized when the humic substances, humic and fulvic acid, have been isolated as single, individual entities, ignoring the issue of their being real or fake entities.

Many chemical and physical methods are now available for fractionation of humic substances. The chemical methods are generally based on the application of differences in some physico-chemical properties, such as differences in solubilities in a chemical reagent, charge characteristics, differences in adsorption, density, particle size, and molecular weight. The physical methods of importance are filtration and centrifugation. Swift (1985) has presented an excellent account of the significance and merits of several of these methods. The methods of fractioning discussed below do not apply only to subdividing humic acids; by changing and selecting the proper parameters, they can be adapted for use in fractioning fulvic acids.

Differences in solubilities of humic acid in chemical reagents, such as in ethanol and in neutral salt solutions, are the basis for fractioning humic acid into different subtypes or subfractions. Fractioning on the basis of charge characteristics is conducted by the electrophoretic technique and ion exchange method. The procedures of fractioning using differences in adsorption vary widely from methods using anion exchange resins to gel chromatography, which applies other types of adsorption resins. The selection of the resins has been discussed earlier, and the purpose for use of a specific resin also varies from one to another resin. For example, anion exchange resins are chosen for attracting negatively charged humic compounds, as indicated before. Other adsorption resins are applied for attracting noncharged humic molecules as in gel chromatography, and fractioning them into different molecular sizes or into different molecular weight size measurements.

6.3.1.1 *Filtration Techniques*

As indicated above, fractionation of a humic substance can also be achieved by physical means. One of the most frequently applied methods is ultrafiltration, using filters with varying pore sizes. Filters generally separate on the basis of linear sizes or dimensions and not necessarily by molecular sizes, and many people use size measurements as identical to molecular

weight sizes, which is scientifically incorrect. In pure colloid chemistry, colloidal sizes can be expressed in terms of linear dimensions (μm or nm) or in terms of mass using molecular weights or daltons. The choice depends on the purpose of the study, and no direct statistical conversion of linear sizes into mass units is available. However, a few daltons are usually considered equivalent to a diameter of 1 nm. Generally, the size of colloids represents a continuum, ranging from 0.001 to 1.0 μm in diameter (Ranville and Schmiermund, 1998), hence defining upper and lower size limits of colloidal particles is arbitrary. Although a size limit of 0.45 μm is most widely accepted, Ranville and Schmiermund (1998) believe that the range of 2 to 5 μm would better describe the hydrodynamic behavior of large colloids, which coincides with the usual clay–silt boundary (2 μm) in soil science. The most common filters used for concentrating of humic matter have pore sizes of 0.45 μm based on the assumption that humic colloids are at the size range of <0.45 μm (Gaffney et al., 1996b). Fractioning can now be carried out by a filtering process using a series of filters varying in pore sizes. This will be discussed below in more detail in fractioning by filtration.

6.3.1.2 Fractioning by Dissolution

Fractioning is based on the capacity of a chemical reagent to break down the humic molecule into several subfractions with different solubilities. A variety of reagents have been used for this purpose; however, in general, most of them function through a dissolution process producing soluble and insoluble humic fractions. For example, humic acid can be fractionated into two fractions by shaking in ethanol. The dissolved fraction is called humatomelanic acid, whereas the undissolved part is called α -humic acid.

As indicated in Chapter 2, by shaking in neutral salt solutions, e.g., 1% NaCl or KCl, humic acid can also be divided into a brown (soluble) fraction and a gray (insoluble) fraction. This process is called by Swift (1985) *salting out*. In soil chemistry, salting out is used for suppression of the double layers, composed of counterions surrounding the charged surfaces of colloidal particles in suspension. The presence of thin double layers causes precipitation of the colloidal particles, and no fractionation of the colloids has in fact taken place.

6.3.1.3 Fractioning by Gel Chromatography

This is perhaps a simple and relatively effective method in achieving molecular fractionation of both fulvic and humic acids, called today size

exclusion chromatography (SEC). The variety of resins available for use has been discussed earlier, and some of them are suitable for fractioning, e.g., Sephadex, Sephagel, silica gel, and the like, whereas others are not, e.g., XAD resins. As an example, the method using Sephadex, synthetic cross-linked polydextranes (Pharmacia Fine Chemicals, Uppsala, Sweden), will be explained below. Sephadex is produced and was introduced in 1959 by Pharmacia Fine Chemicals in Uppsala, Sweden, after the development of the gel filtration technique by Forath and Flodin (1959). The analysis of gel chromatography with Sephadex is more a method of filtration; hence, the method is also given the name *gel filtration*. A column of swollen Sephadex gel beads is prepared, using bead sizes commonly of between 20 and 300 µm, and a determined amount of a humic acid solution is then placed on top of the column. As soon as it has drained into the column, it is eluted with distilled water at a controlled flow rate of 40 mL/h. The subfractions collected are purified and freeze-dried. This gel filtration procedure can be repeated several times until sufficient subfractions are collected for use in analysis and research (Tan, 1977; Tan and Giddens, 1972). The filtration is diagrammatically illustrated in Figure 6.2. The pores between and within the Sephadex gel beads act as a chromatographic medium. The large open circles in the figure are the Sephadex beads. The small and large black dots represent small and large molecules of humic acids, respectively. This mixture of humic molecules in solution is moving through the porous column of Sephadex beads. The larger humic molecules tend to flow through the spaces between the beads, hence move quickly through the column (see Figure 6.2). On the other hand, the smaller humic molecules also enter the pores within the beads and this process of diffusion makes them move much more slowly through the column than the large molecules. This is then the reason why they are eluted last, as shown in Figure 6.2. Consequently, the elution curve may be characterized by two peaks: (1) the first peak representing the high molecular weight fraction and (2) the second peak representing the low molecular weight fraction.

6.3.1.4 Starches, Silica, and Other Gels versus Sephadex

Starches and silica gel were used in chromatography long before Sephadex was invented. The latter, being a cross-linked dextran, is in fact also a starch-based resin. They can all be used as the gel bed in column chromatography, but starch and silica gel are also often applied to plastic sheets, glass plates, or aluminum in thin layer chromatography. Different particle

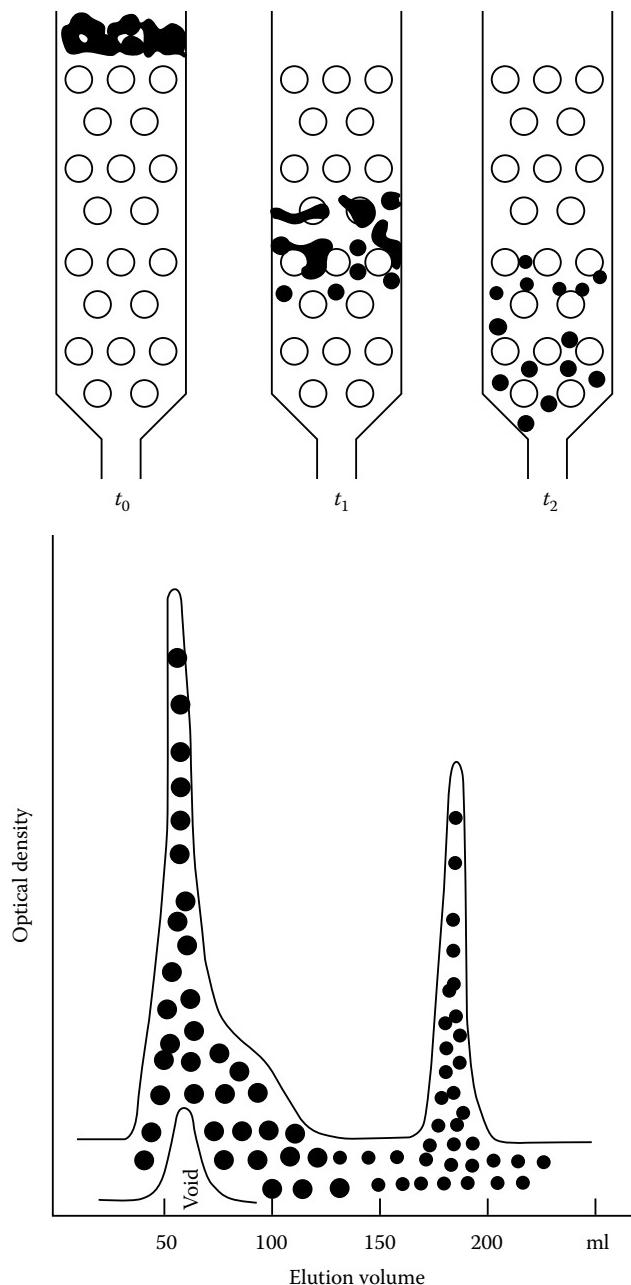


Figure 6.2 (Top) Schematic diagrams of Sephadex gel filtration, called today size exclusion chromatography (SEC) of humic acid into a high molecular weight (●) and low molecular weight fraction (○); (○) Sephadex beads. (Bottom) Elution curve characterized by two peaks, a peak representing the high molecular weight fraction and a second peak representing the low molecular weight fraction.

sizes are used depending on the purpose of separation by molecular sizes. In the case of silica gel, a particle size of 40–60 µm is most commonly applied in column chromatography. Because of its polar nature, nonpolar fractions are eluted first and the polar fractions tend to be eluted last in chromatography using silica gels.

Other reagents occasionally used as the gel substrate are polystyrene, polyamides, aluminum oxides, cellulose, and glass beads. However, the most frequently used gel materials today are the cross-linked polymers of dextran, polysaccharides, polystyrene, and the like, distributed under various generic names such as Sephadex, Sephagel, Biogel, and Cellogel. Other types related to Sephadex are currently also available, e.g., Sepharose, Sephacryl HR, and Superdex (Wu, 1999; Sharma, 2007). Sepharose is made from agarose, and Sephacryl is a covalent cross-linked dextran, whereas Superdex is also composed of cross-linked agarose but to which dextran is covalently bonded.

The separation of humic substances into different particular molecular sizes depends on the size exclusion limit of a specific type of Sephadex used. Different types of Sephadex resins with different molecular weight cutoffs can be used separately or in sequence (tandem), e.g., Sephadex G-50, G-20, and G-10, with molecular weight cutoffs of 30,000, 3000, and 1000, respectively. Sephadex resins with lower molecular weight cutoffs than 1000 are now available. A more complete updated list of the various types of Sephadex showing their cutoff limits are shown in Table 6.3. For more details, reference is made to Tan (1998) and the *Handbook for Sephadex-Gel Filtration in Theory and Practice, Pharmacia Fine Chemicals*, Uppsala, Sweden (1969).

6.3.1.5 Size Exclusion Chromatography, LPSEC, and HPSEC

As indicated above, the gel filtration method with Sephadex gels is called now size exclusion chromatography (SEC). It can be distinguished into LPSEC and HPSEC, which will be explained below. They need apparently a closer examination due to the popularity and great importance given by many scientists in humic acid research today. The method, allegedly developed in 1955 by Grant H. Lathe and Colin R. Ruthven at the Queen Charlotte Hospital, London, used starch as the gel bed (Lathe and Ruthven, 1955). In 1959 Jerker Forath and Per Flodin applied dextran (Forath and Flodin, 1959), and Sephadex was born.

The method first called gel filtration using Sephadex, described in the previous section in some detail, was later named low-pressure size exclusion

Table 6.3 Types of Sephadex and Molecular Weight Cutoff Limits and/or Fractioning Ranges

| <i>Sephadex</i> | <i>Fractioning Range</i> |
|-----------------|--------------------------|
| G-10 | 1000 |
| G-15 | 1500 |
| G-20 | 3000 |
| G-25 | 5000 |
| G-50 | 30,000 |
| G-75 | 80,000 |
| G-100 | 4000–100,000 |
| G-150 | 5000–300,000 |
| G-200 | 5000–600,000 |

Source: <http://en.wikipedia.org/wiki/Sephadex>, accessed September 10, 2013; Sharma, B. K. Instrumental methods of chemical analysis. Introduction to analytical chemistry. In: *Chromatography*, Fifth Edition, M. Sharma, and A. Sharma (eds). GOEL Publishing House, Meerut, India, 2007; Wu, C. (ed.). *Column Handbook for Size Exclusion Chromatography*. Academic Press, San Diego, CA, 1999.

chromatography or LPSEC, because the humic solution is allowed to flow through the bed column by gravity only, and no additional forces, higher than the gravitational force, are applied. On the other hand, in HPSEC or high-pressure size exclusion chromatography, pressure usually between 50 and 350 bar is used to move the solution through the column. Gel beds are also built with materials of smaller particle sizes (2–5 µm), providing HPSEC with a better resolving power in fractioning humic acids. Recently an ultra-high performance size exclusion (UHPSEC) method has been reported, capable of increasing the speed of analysis allegedly about 10 times and at the same time enhancing the resolving power considerably (Janco et al., 2013).

In the new models, HPSEC instruments are also equipped with detectors, for example, with a UV-visible light spectrometer. Commercially ready-made columns tailored to the preferences for a particular brand of gel are also available today. Both the terms “low pressure” and “high pressure” have lately been changed, and the methods are now called *low performance* and *high performance* SEC, respectively.

6.3.1.6 Fractioning with Anion Exchange Resins

The method of fractioning applying anion exchange resins is in fact closely related to gel chromatography discussed above. Anion exchange resins have been tried several times in subdividing humic substances (Wright and Schnitzer, 1960; Roulet et al., 1963; Barker et al., 1967). The adsorbed humic substance is eluted with a buffer or salt gradients, applied at gradually increasing or decreasing concentrations. However, what exactly the subdivisions are is still not clear. A subdivision by molecular weight size was supposed to be the objective, but anion exchange functions through attraction by electrical charges. What then is the salient feature of subdividing a humic molecule on electrical charge properties? Swift (1985) shows a fulvic acid eluted by a salt (Tris + NaCl) gradient, yielding an elution curve with two peaks. The first peak is produced by elution with the Tris reagent, and the second peak is the result of elution with NaCl. However, Swift fails to explain whether the peaks represent fractions with different sizes or the like, or whether they are two subfractions of fulvic acid different in electrical charges. How are differences in molecular weights related to differences in electrical charges? These questions have to be answered before one can conclude that ion exchange media lend themselves very readily to fractionation of humic substances.

6.3.1.7 Fractioning by Filtration

Two general types of filters are available for this purpose, e.g., membrane filters and depth filters. The membrane filters, such as cellulose acetate, cellulose nitrate, and silver membrane filters, function by sieve action. All humic particles larger than the filter's pore size will be retained. Depth filters, e.g., glass fiber filters, also function as sieves, but the sieve action is caused by a matrix of fibers forming a labyrinth of meandering flow channels with variable pore sizes. These fiber filters are sometimes noticed to pass particles larger than their pore size. The main disadvantage with all filters is the problem of clogging when large amounts of humic material are collected, requiring the use of adequate suction. For the merits of the various filters with respect to pore size, chemical composition, and flow characteristics, reference is made to Aiken (1985). Fractionation of a humic substance is achieved by using a series of filters of different pore sizes, either in ascending or decreasing order of size limits. By using only one filter size, the process will only concentrate the fraction of the humic substance above the pore size cutoff limit.

6.3.1.8 Issue of Linear versus Molecular Weight Sizes in Filtration

Although the filtration technique is, by design, to be used for separating the humic substances according to linear size limits, in the 1990s the method was expanded to sort out humic substances into different molecular weight fractions by using several filters. Membrane filters have been made available with pore sizes from several micrometers (μm) to several nanometers (nm). Filters with larger pore sizes are used for sieving relatively larger size particles, and the process is called *microfiltration*. On the other hand, filters with the smallest pore sizes can be applied for filtering molecules in solution, and this is called *ultrafiltration*.

However, linear sizes have been discussed before as not being equivalent to molecular weight sizes, and a few daltons are taken for convenience to equal 1 nm. Tan and McCreery (1975) note that degree of polymerization of humic substances affects their linear sizes as well as their molecular weights. The data of the authors summarized in Table 6.4 demonstrate the relation between size of a molecule and its molecular weight. By assuming that the humic molecules are spherical, the larger the size of the sphere, the larger will be the numerical value of the molecular weight of humic acid. It is true that other factors, e.g., density, will spoil this relationship, while many scientists may also disagree that humic substances are spherical; however, until a better concept can be advanced, the hypothesis of the authors above seems to solve the issue satisfactorily for the time being. Most scientists are using

Table 6.4 Molecular Weights and Sizes (in Å and nm) of Humic Acid Fractions Obtained by Sephadex Gel Filtration

| <i>Molecular Weight</i> | <i>Molecular Volume</i> | <i>Radius</i> | |
|-------------------------|-------------------------|---------------|------|
| | Å | Å | nm |
| 30,000 | 23,622 | 17.8 | 1.78 |
| 5000 | 3937 | 9.8 | 0.98 |
| 1500 | 1181 | 6.6 | 0.66 |
| 1000 | 787 | 5.7 | 0.57 |

Source: Tan, K. H. *Environmental Soil Science*, Second Edition. Marcel Dekker, New York, 2000; Tan, K. H., and R. M. McCreery. Humic acid complex formation and intermicellar adsorption by bentonite. Proc. Int. Clay Conf. Mexico City, Mexico, July 16–23, 1975, S. W. Bailey (ed-in-chief). Applied Publ. Ltd., Wilmette, IL, pp. 629–641, 1975.

linear size limits interchangeably with molecular weight limits anyway, as can be noticed from the discussion below.

Membrane filters are now available with ultrafine pores, assumed equivalent to molecular weight cutoff limits between 50 and 1 million. Such an ultrafiltration technique was introduced by Lobartini et al. (1997) using an Amicon cell model 8050 adapted for use with a continuous flow. The method, employing a series of cellulose membranes, starts with a membrane with a 10,000-Da exclusion limit. A humic solution is placed in the cell, and sufficient amount of distilled water is passed with the aid of a peristaltic pump until the solution flowing from the cell is totally colorless. The fraction passing through the filter is the fraction with a molecular weight of <10,000 Da. The following fractions are then obtained by treating the remaining HA (m.w. >10,000) in the Amicon cell with the same procedure but by using at each step membranes of different exclusion limits in order to yield fractions with molecular weights between 10,000 and 30,000, 30,000 and 50,000, 50,000 and 100,000, and >100,000 Da. Each fraction is purified and concentrated by passing through a column of XAD-8 resin.

6.3.2 *Fractionation of Fulvic Acid*

Because of the small size or molecular weight size of fulvic acid, its fractionation has attracted less research attention than that of humic acid. Only a limited number of attempts have been reported for separating fulvic acid further into subfractions. The methods at our disposal for this purpose are, in essence, similar to the chemical and physical methods used for fractionating humic acid.

Sephadex gel filtration has been reported to yield two fulvic acid subfractions, a high molecular weight and a low molecular weight fraction (Tan, 1998; Khan and Schnitzer, 1971). However, Forsyth (1974) claims to have subdivided fulvic acid into four fractions, A, B, C, and D, with activated charcoal. By using a different procedure, Stevenson (1994) seems to support the separation of fulvic acid into two fractions only. The acid supernatant, following separation of humic acid by centrifugation, is transferred by the author above into a dialysis bag and dialyzed against distilled water. After equilibration for three to four nights, both the dialysate (colored water) and the nondialyzable fraction within the bag are collected and separately purified and concentrated with the XAD-8 resin technique. The purified nondialyzable fulvic fraction is called by Stevenson (1994) the high molecular weight generic fulvic acid, whereas

the purified dialysate is called by the author above the low molecular weight generic fulvic acid.

Waksman (1936) also claimed in the early days to have separated fulvic acid into two fractions. By adjusting the pH of the fulvic acid solution to 4.8, after the humic acid fraction was removed by acidification and centrifugation, a precipitate is produced that he called β -humic acid. As discussed before in Chapter 2, the correctness of such fractionation is open for discussion.

Today fractionation of fulvic acid into several subfractions is made possible by the availability of filters with pore sizes ranging from 1 to 450 nm (0.001–0.45 μm). The process, applying several of these ultrafilters with very small pore size limits in ascending or descending order, earlier called ultrafiltration, has not been exploited with fulvic acid.

6.4 Aquatic Humic Matter

6.4.1 Extraction Methods

As discussed above, a different approach has to be taken in extraction of aquatic humic matter. The process has to take into account concentrating the humic colloids in solution that are generally present not only in small concentrations, but also with a composition dominated by fulvic acids, the smallest humic substances by size or molecular weight. A variety of methods, including physical and chemical methods, have been discussed previously, and according to Aiken (1985), it is recommended to employ a variety of methods, rather than a single method alone, if a high-quality product is to be produced. The author above cited four steps for obtaining the highest quality material: (1) filtration through a micropore filter with a pore size of $\leq 0.45 \mu\text{m}$, (2) concentration by adsorption on XAD or Duolite A-7 resin, (3) isolation from inorganic and organic contaminants, and (4) preservation by freeze-drying. Step 3 is, however, stated in the wrong context, since removal of inorganic and organic contaminants is a purification and not an isolation issue.

6.4.1.1 Extraction by XAD-8 Gel Filtration

This is a chemical method recommended by the IHSS. The method was first presented by Thurman and Malcolm (1981) and later adopted as the procedure of the IHSS (Aiken, 1985). It is reported to be capable of extracting aquatic humic matter from a number of surface and groundwater samples

with a DOC as low as 0.7 mg C/L. Tan et al. (1991) have used it for extraction of humic substances in humic (black) water of streams and swamps in southeast Georgia, United States, with an average DOC content of 10.0 mg/L.

The IHSS method requires the construction of a properly prepared XAD resin column, which is prepared as follows. Amberlite XAD-8 resin (40–60 mesh; Rohm and Haas) is cleaned by shaking it successively in 0.1 M NaOH and ethanol. The resin is rinsed several times with distilled water before it is loaded into a polyethylene chromatographic column. After leaching alternately with 0.1 M NaOH and 0.1 M HCl as specified by Thurman and Malcolm (1981), the column is ready for use. Five to ten liters of humic (black) water are then filtered through a micropore filter with a pore size of 0.45 µm to remove suspended inorganic matter. The filtered water is acidified with HCl to pH 2.0 and allowed to flow by gravity through the prepared XAD-8 column at a flow rate of 200 mL/h. The humic substances retained by the column are washed thoroughly with distilled water and eluted with 0.2 M NaOH. The dark-colored solution is collected and acidified with HCl to pH 1.0, to precipitate the humic acid from the solution. The humic acid precipitate is separated from the fulvic acid that remains in solution by centrifugation at 10,000 rpm for 15 min. After collection, the humic acid is then purified by twice redissolving, reprecipitating, and centrifuging. The final precipitate is then washed once with a very dilute HCl + HF mixture, dialyzed against distilled water, or subjected to a final purification with a hydrogen-saturated cation exchange procedure as discussed earlier.

The FA in solution is concentrated by filtering again through the XAD-8 column. The adsorbed FA is washed twice with distilled water and eluted with 0.1 M NaOH, after which it is allowed to flow through an H-saturated cation exchange (Dowex 50-X8) column for a final purification. The purified FA can be used for further analysis as such, or it can be freeze-dried, weighed, and stored in an amber-colored flask.

6.4.1.2 Extraction by Freeze-Drying

This is a very simple physical method for the collection of aquatic humic matter. It has been used by Tan et al. (1990, 1991) in the extraction and isolation of humic substances in humic water of streams and swamps from southeast Georgia, United States. By this method, a determined amount of humic water is filtered by microfiltration (0.45 µm) and freeze-dried to concentrate and collect the humic matter. The humic and fulvic acid is then extracted and isolated from the freeze-dried sample by the 0.1 M NaOH

Table 6.5 Humic (HA) and Fulvic Acid (FA) Content in Humic Water of Streams in Southeast Georgia, United States, Extracted by the XAD-8 Resin and NaOH Freeze-Dry Methods

| Humic Water (Georgia, USA) | XAD-8 Resin | | | 0.1 M NaOH | | |
|-------------------------------|-------------|-----|-------|------------|-----|-------|
| | FA | HA | FA/HA | FA | HA | FA/HA |
| | g/kg | | | g/kg | | |
| Okefenokee Swamp | 720 | 280 | 2.6 | 675 | 375 | 1.8 |
| Satilla River | 726 | 274 | 2.6 | 600 | 400 | 1.5 |
| Ohoopee River | 725 | 275 | 2.6 | 696 | 304 | 2.3 |

Source: Tan, K. H. et al., *Comm. Soil Sci. Plant Nutr.*, 21, 1999–2016, 1990; Tan, K. H. et al., *Comm. Soil Sci. Plant Anal.*, 22, 861–877, 1991.

Note: The term *black water* has been changed in this book to *humic water* in accordance to new ideas in aquatic organic matter science (see chapter 1 of Tulonen, 2004).

procedure as described above. Purification, considered an integral part of the extraction procedure, is conducted as described earlier. As can be noticed from the data in Table 6.5, this method yields the same amounts of humic matter as the IHSS-recommended XAD-8 resin technique. No significant differences can be noticed in the total (combined) concentrations of humic acids and fulvic acids extracted between the two methods, indicating that both methods are equally suitable for extraction and isolation of aquatic humic matter. However, a considerable difference can be noticed in the proportional composition of fulvic acid and humic acid extracted from the aqueous medium. The humic acid content obtained by freeze-drying appears to be invariably larger than that extracted by the XAD–resin method. This is clearly reflected by the FA/HA ratios.

6.4.2 Fractionation of Aquatic Humic Matter

The opinion exists that fractionation techniques for aquatic humic substances have not been developed to the same extent as their concentration and isolation techniques (Leenheer, 1985). Such a statement is out of context since a great variety of fractionation techniques can be adopted from terrestrial humic acids. The issue with aquatic humic substances is that they are present in very low concentration; hence, fractionation is only possible after the aquatic humic substances have been isolated and collected properly in adequate amounts. Concentration and isolation techniques are the first

steps for subdividing aquatic humic matter. Now that efficient procedures are available for concentration and isolation of aquatic humic substances, fractioning of these compounds is expected to be a relatively simple matter. Contradicting his statement above, a variety of chemical and physical fractionation methods are nevertheless cited by Leenheer (1985), such as precipitation methods, solvent extraction, adsorption chromatography, electrophoresis, ultrafiltration, and ultracentrifugation. This list is identical to, although shorter than, the fractionation procedures discussed earlier for terrestrial humic and fulvic acids. However, the author's discussion on each of the methods leaves us wondering what the purpose is for listing the methods at all, if they are considered unsuitable. For example, the precipitation method, by adding metals or inorganic and organic acids, is according to the author above a very crude method, and is more suitable for isolation and concentration purposes. Leenheer (1985) also believes that partitioning of aquatic humic substances by solvent extraction is not possible because of their amphipathic character that will form dark films at the liquid–liquid interfaces. The author seems not to realize that humic acid can be subdivided into hymatomelanic acid and α -humic acid by dissolution in ethanol. At present, a number of effective fractioning procedures have been developed for terrestrial humic matter. The present author can see no reason why both humic acid and fulvic acid from the aquatic environment cannot be fractionated by the same physical and chemical procedures created for terrestrial humic matter.

6.4.2.1 Fractionation by Ultrafiltration

As indicated in preceding sections, the development of filters with pore sizes $<0.45\text{ }\mu\text{m}$ makes it possible to extract, isolate, and fractionate the humic substances in soils and water. An example of fractionation of humic acids by ultrafiltration using flatbed membrane filters will be provided in this section.

For fractionation of aquatic fulvic acid, a series of filters with very fine pore sizes is recommended. Hence, methods using filters with pore size limits from 0.001 to $0.0025\text{ }\mu\text{m}$ should be able to produce fulvic acid fractions partitioned by molecular weights from 1000 to 30,000 Da. Additional research has to be conducted to verify this contention. From Sephadex G-50 (fractionation range = 1500–30,000 Da) gel filtration, indications have been presented that fulvic acid can be subdivided into two fulvic acid fractions (Tan, 1977; Khan and Schnitzer, 1971).

More recently, the use of hollow-fiber ultrafilters has been suggested for fractionation of aquatic humic and fulvic acids by a couple of scientists from the American Chemical Society (Gaffney et al., 1996a). Hollow-fiber ultrafilters are now available, capable of size fractioning humic compounds from 1 to 450 nm (0.001–0.45 µm). These filters are considered to differ from the flatbed filters in that they prevent *polarization* of the filter when large volumes have to be processed. What the authors mean by polarization is piling up of the (filtered) trapped humic molecules on a flatbed filter during analysis of large volumes of water. The trapped molecules are believed to act as filters themselves, and smaller substances can be trapped in the pores of the layered larger molecules. This polarization effect is said to be prevented by using a hollow-fiber filter, because water is pumped from the inside to the outside of the filter during filtration, causing a flow of water parallel to the hollow-fiber filter. By first using hollow-fiber filters for fractioning fulvic acids to molecular size fractions down to 1 nm (equivalent to molecular size limits of 1000), these humic compounds can be fractionated further by using flatbed filters down to molecular weight sizes of 500.

From the discussions above and those in earlier sections, the question can be raised whether fractionation by ultrafiltration will yield legitimate fractions differentiated by molecular sizes, since it appears that the method would yield any molecular weight fraction as imposed by any exclusion limits used in the procedure. Unfortunately, no definite answer can be given at the moment, and more research has to be conducted to solve this issue. However, in consolation, it can perhaps be stated that the presence of a seemingly endless variety of molecular fractions concurs with the condition in a natural ecosystem where decomposition at all stages and new synthesis of humic substances are taking place in a never-ending process.

6.5 Determination of DOC and DOM

Dissolved organic matter, known in particular by its acronym DOM, has attracted lately a great deal of attention in aquatic organic matter research. The issue as an operational compound has been discussed in detail in Chapter 1, Section 1.4. As examined earlier, the term DOM is unfortunately considered synonymous with DOC by geologists, hydrologists, or geochemists of the U.S. Geological Survey (Leenheer and Croué, 2003; Wershaw et al., 2005), which is not only very controversial but brings confusion to the basics of organic chemistry. Because of the huge attention directed by geologists toward DOM, it is perhaps necessary to reinforce what was

discussed in Chapter 1 that in analytical chemistry, organic matter is considered to contain, on average, 58.0% organic carbon. Hence in terms of DOC, the amount of “aquatic organic matter” is considerably less than DOM by a factor of 1.724, called the *van Bemmelen factor* (Tan, 2005). Soil organic carbon is commonly determined by dry combustion methods, carbon analyzers, or by the Walkley–Black titration method (Tan, 2005). However, these methods are not capable of analyzing the very small amounts of DOC normally present in natural waters, unless huge volumes of water are evaporated first to concentrate and accumulate sufficient amounts of samples for analysis of DOC. Procedures for extraction of carbon from organic matter fractions, such as from carbohydrates, lignin, and other biopolymers, are not available. However, at the turn of the century, a method of microanalysis was published by the Limnology Department of Uppsala, Sweden, showing promise for the direct measurement of DOC, which is discussed below.

6.5.1 Determination of DOC

A method of microanalysis of DOC directly from water has recently been reported by Bertilson and Tranvik (2000). The ingenious procedure indicating injection of water samples into a carbon analyzer is a technique used similarly in gas–liquid chromatography. For ease of reading and enhancement of comprehension, the procedure is presented by the present author below in a revised or modified form. It is offered with the purpose that other researchers may pursue the DOC analysis further with the hope to develop it into an acceptable standard procedure so that the controversy of using interchangeably the terms DOC and DOM can be resolved.

Lake water is collected by the authors above and prepared for analysis by first purifying it repeatedly (four times) by filtering using apparently a commercial “household” ultrapure Millipore water filter. Aliquots of 5 mL filtered lake water are then acidified with 20 μ L of 2 M HCl solutions and purged with CO₂-free air for 5 min to remove inorganic carbon, which is presumably made up of mainly CO₂ gas in solution. Samples of 33 μ L acidified purged water are then injected into a Shimadzu Total Carbon Analyzer instrument, model TOC-5000, equipped with an infrared detection device. The instrument uses a high-temperature catalytic oxidation system for the determination of DOC. Organic carbon concentration is calculated using standards prepared from potassium hydrogen phthalate and analyzed similarly to the water samples. The effectiveness in removing the inorganic carbon is checked by analysis of inorganic carbon in purged and nonpurged samples.

6.5.2 Extraction and Determination of DOM

Since DOM is considered very important by prominent geologists, limnologists, and/or hydrologists in the maintenance of healthy ecosystems and in aspects of humus chemistry, it is perhaps of interest, and/or for completeness, to examine its extraction and determination from aquatic systems. The extraction procedure is also not immune from controversies. The pore size of filter used varies from 0.45 µm as reported by Leenheer and Croué (2003), to 0.22 µm and even as large as 63 µm as suggested by Wershaw et al. (2005), whereas other information available in the literature recommends the use of a fiber glass GF/F filter. This issue on filter sizes has been examined in some detail in Section 6.3.1.1. Perhaps it is helpful to consider that the most common filters used for concentrating of humic matter have pore sizes of 0.45 µm based on the assumption that humic colloids are at the size range of <0.45 µm (Gaffney et al., 1996). Aquatic humic matter makes up part, if not the bulk, of DOM and will be extracted together with DOM. The isolation procedure discussed below is an adaptation of the methods suggested by Leenheer and Croué (2003) and Wershaw et al. (2005). Explicit step-by-step instructions of the analytical procedure are absent in Leenheer's and Wershaw's papers, as well as in other sources in the literature, and the method of isolation of DOM below is written by connecting bits and pieces found scattered throughout the papers.

Samples of natural waters are collected and preferably filtered using a 0.45 µm pore size membrane filter. The total aquatic organic matter content in the water sample, given the name TOC by the authors above, is then divided into (1) a DOM fraction, the fraction that passes through the filter, and (2) a POM or particulate organic matter fraction, which is retained by the filter. The DOM fraction passing through the 0.45-µm filter is divided again into a humified and nonhumified portion by analysis with the XAD-8 resin method as described earlier in Section 6.4.1.1. Humic substances are retained by the XAD-8 resin, whereas the nonhumified part that passes through, in other words “not sorbed by the resin,” is called the *true DOM*. This true DOM material is subdivided further by sequential sorption on XAD-8 and XAD-4 resins according to its polarity features into (1) HDOM, hydrophobic DOM or the least polar DOM, presumably adsorbed by XAD-8; (2) TDOM or transphilic DOM, a more polar DOM, adsorbed by XAD-4; and (3) HDOM or hydrophilic DOM, the most polar component. The acronyms have been changed by the present author to provide a more uniform and systematic system of nomenclature. A flow sheet, looking more like a “family tree,” is also provided by Leenheer and Croué showing the tier of classes of DOM and its fractions. It

is perhaps apparent from the discussion above that many concerns are raised on the operational concept of DOM, which is reflected by the authors' multiple statements regarding this aspect in their journal papers. This controversy becomes even more obvious when studying their flow sheet, in which the names DOM and DOC are also used interchangeably. Therefore, readers interested in further details are referred to the particular papers on these issues.

6.6 Types of Humic Substances

Most scientists consider humic acid and humic acid fraction (or fulvic acid and fulvic acid fractions) as identical. Through the whole literature, these terms are used interchangeably, although *types* and *fractions* usually carry different connotations. The type is the original humic compound, and this can be broken down into several fractions. Hence, the names humic acid and fulvic acid are, in essence, referring to the types of the humic substances. These two compounds, regardless of their being real, artifacts, or operational compounds, are neither obtained by fractioning of the humic substances nor are they fractions of humic acid or fulvic acid, respectively. As indicated before, humic matter is composed of a mixture of humic substances, and humic acid and fulvic acids are two of the humic substances making up the mixture. This problem can be illustrated again perhaps by using the analogy with humus, which is a mixture of carbohydrates, protein, lignin, nucleic acids, enzymes, humic matter, etc. The carbohydrate, protein, or lignin is the type of component making up the humus, and it is rather confusing to realize that a carbohydrate fraction or protein fraction infers the respective component itself. The analogy can be illustrated further by using clay as an example. The soil's clay complex is composed of a variety of clay minerals, e.g., kaolinite, halloysite, smectite, vermiculite, and chlorite. Kaolinite is one of the types of clay and is not considered a kaolinite fraction of the clay complex. It is true that in both cases, the use of the term *humus fraction* or *clay fraction* is often used and admissible; however, its application then carries a different meaning.

With this in mind and in view of the discussion in the preceding sections, three major types of humic substances can be recognized, which is unfortunately not much different from the categories in the old theories. Each of the three types can be fractionated into several fractions. The concepts of these three types of humic substances are apparently valid for terrestrial, aquatic, peat, geologic, and anthropogenic humic matter. At the present time, no information is available for the presence of other types of humic compounds

due to differences in origin or method of extraction. Speculations on the presence of differences between terrestrial and aquatic humic acids have off and on been presented.

Recently, a humic substance called kerogen has been brought to attention as a very specific type of humic acid present only in aquatic environments. As stated earlier, it is considered by some as a condensed form of aquatic humic acid with diagenesis as the mechanism for its formation. A classification scheme for kerogen has even been proposed distinguishing it into types I, II, and III (Vandenbroucke et al., 1985). Type I kerogen is found in lacustrine deposits, whereas type II kerogen is typical for marine shales. Type III shows the influence of the continents. However, at the present state of knowledge, very little is known about kerogen and no information has been presented about its extraction, isolation, fractionation, and characterization.

In line with the divisions of kerogen, perhaps terrestrial humic acid can also be distinguished on the basis of the monomeric type of lignin, e.g., softwood humic acid, hardwood humic acid, and grass or bamboo humic acid. However, all of the above needs to be investigated in further detail and some kind of definite evidence has yet to be provided.

Substances labeled copropel, sapropel, förna, dy, pelogoea, and dopplerite have at times also been considered major types of aquatic and marine humic acids (Swain, 1963; Stevenson, 1994), although some geochemists tend to use them for subdivisions of humic substances at lower categories. Subclasses of copropelic humic acid, sapropelic humin, and the like have been suggested by Hatcher et al. (1985) and Breger (1976). The potential is present for recognition of these aquatic or marine types of humic acids; however, it appears that more research data are required showing similarities and differences in properties and behavior in order to establish their qualifications as members of the types of humic substances.

As summarized in Table 6.6, the present author will focus on the three established types of humic substances, differentiated by their solubilities in

Table 6.6 Three Major Types of Humic Substances Differentiated by Differences in Solubility in Acids, Alkalies, and Water

| Type of Humic Substance | Alkali | Acid | Water |
|-------------------------|-----------|-----------|-----------|
| Fulvic acid | Soluble | Soluble | Soluble |
| Humic acid | Soluble | Insoluble | Insoluble |
| Humin | Insoluble | Insoluble | Insoluble |

basic and acidic solution. Each will be discussed in more detail below, followed by their fractions. The copropellic and sapropellic substances and the other aquatic and marine counterparts will also be covered but in less detail.

6.6.1 Fulvic Acid

As noticed from Table 6.6, fulvic acid is the type of humic compound that is soluble in alkali, acid, and water. It is assumed to be a colloidal polydispersed, amorphous humic substance with yellow to brown-black color. Fulvic acid exhibits hydrophilic and highly acidic properties, and of the three major types of humic substances it is relatively the smallest in molecular size, ranging from a few hundred to a couple thousand atomic units or daltons. The number-average molecular weight is reported to range from 175 to 3570 (Schnitzer and Skinner, 1968). Fulvic acid is believed to be the agent of major importance in the mobilization of sesquioxides in the podzolization process (Forsyth, 1974). Freeze-dried fulvic acid is sometimes difficult to dissolve in water because of polymerization of the compound during freeze-drying. The freeze-dried material is often electrostatically charged since the fine fulvic acid particles are attracting themselves to a metal or plastic spatula. Fulvic acid will decompose upon heating without burning, but through a gradual charring process. It is reported to start burning at 190–200°C if an oxidizing agent is present (Orlov, 1985; Hoffman and Schnitzer, 1968). Because of this and since irreversible changes are noticed to occur at 50°C, drying fulvic acid is suggested to be carried out at 35–40°C in vacuum over P_2O_5 .

Fulvic acid is often ignored and has received intermittent research attention. It was perhaps the first humic compound that was extracted in the early days; however, research interest dropped considerably in the 1960s–1970s. In Stevenson's (1994) opinion, this is due to difficulties in recovering fulvic acid from the acidified soil extract following separation of humic acid. The remaining acidic soil extract contains substantial amounts of inorganic contaminants and NaCl salt as a result of acidification of the NaOH extract with HCl. The author above believes that removal of these contaminants always results in considerable losses of fulvic acid. However, the attention to fulvic acid skyrocketed again with the discovery of aquatic humic matter. In aquatic environments, fulvic acid is the dominant type of humic matter. Depending on which authority to believe, fulvic acid is considered either a precursor for formation of humic acid or a degradation product of humic acid. This will be discussed in more detail below in the section on bioformation of humic substances.

6.6.1.1 Fulvic Acid Fractions

6.6.1.1.1 High Molecular Weight Fraction

This is the fulvic acid fraction separated by Sephadex G-50 (Tan, 1977). It is the fraction, characterized by a molecular weight >1500 , that is eluted first from the Sephadex column. It makes up approximately 20% to 25% of the total fulvic acid compound.

By applying Sephadex G-10 with a smaller fractionation range (molecular weight cutoff = 700), a fulvic acid fraction is obtained with a molecular weight >700 , called fraction D₂ by Khan and Schnitzer (1971). It makes up 16.5% of the fulvic acid. Fraction D₂ may also include the fulvic acid fraction with a molecular weight range of >1500 as obtained above with Sephadex G-50.

6.6.1.1.2 Generic High Molecular Weight Fraction

This is Stevenson's (1994) version of a high molecular weight fraction of fulvic acid, obtained by dialysis of fulvic acid in a membrane tubing. The fraction retained within the membrane tubing is, after purification by the Amberlite XAD-8 resin technique, called generic fulvic acid. The name high molecular weight is apparently used in a relative sense with respect to the dialysate, a fraction that has passed the membrane tubing and is assumed to be lower in molecular weight. No mention is made of any limit specifying the molecular weight cutoff of the membrane tubing.

6.6.1.1.3 Low Molecular Weight Fraction

This is the fulvic acid fraction that is eluted last from the Sephadex G-50 column. It has a molecular weight in the range of 1500 or smaller. It is the dominant fraction, making up approximately 75% to 80% of the total fulvic acid compound.

By using Sephadex G-10, Khan and Schnitzer (1971) have obtained a fulvic acid fraction with a molecular weight ≤ 700 , called fraction D₁. It is also the dominant fraction and its amount of 83.5% does not differ much from that obtained by Sephadex G-50 filtration. Apparently, fulvic acid fraction obtained by Sephadex G-50 contained fraction D₁.

6.6.1.1.4 Generic Low Molecular Weight Fraction

As indicated above, this is Stevenson's (1994) version of a fulvic acid fraction that has passed a dialysis membrane tubing, and has been purified by the XAD-8 resin method.

6.6.1.1.5 Fractions A, B, C, and D

These are the fulvic acid fractions adsorbed by activated charcoal and separated by successive elution with different reagents (Forsyth, 1947). Fraction A, containing substances eluted by water and 0.1 N HCl, includes sugars and amino acids. It is a colorless or slightly yellow filtrate. Fraction B is the acetone-soluble eluate and is the most deeply colored fraction. It is believed to be composed of tannins or phenolic glycosides. The remaining residue in the charcoal column yields fraction C upon elution with distilled water. It is a colorless eluate that produces a white precipitate upon addition of alcohol. This fraction is believed to contain polyuronides and sugars. Fraction D, the last fraction in the column, is eluted with 0.5 N NaOH. The eluate has a deep red wine color, and is assumed to be rich in nitrogen, organic phosphates, and sugars. According to Tan (1977), only fractions A, B, and D are of importance since fraction C seems to be small in amount and is mostly composed of tailings from fraction B.

Results of infrared analysis by the author above indicate that the tannins identified by Forsyth are most likely to be fulvic acid fractions. The sugars originate perhaps from polysaccharides, since the latter are found to be important components of fulvic acids (Tan and Clark, 1968; Clark and Tan, 1969).

6.6.2 Humic Acid

This is the type of a humic substance that is soluble in basic solvents but insoluble in acidic conditions and in water (Table 6.6). Humic acid is now believed to be present in soils as well as in peat and aquatic environments. A condensed form of aquatic humic acid is called earlier kerogen. At the present state of knowledge, most of the research data suggest that humic acid from aerobic soils is similar to that from anaerobic environments (Hatcher et al., 1981b).

Humic acid is generally characterized by a dark brown to black color. It is an odorless, colloidal polydispersed substance, and assumed to be hydrophilic and acidic in nature. In the moist state, humic acid is said to have a slightly bitter and acid taste (Orlov, 1985). Its molecular weight is very high, ranging from several hundreds to thousands of daltons. Although humic acid is considered to be amorphous, after freeze-drying most of the humic acid particles are rhombic or rhombohedral in shape, like the granular crystals of sugar. They seem to possess high electrostatic charges, since the particles are instantly attracted to a metal spatula, which is especially true with the finer particles.

The humic acid compound is usually low in ash content when the purification process includes treatment with a dilute mixture of HCl + HF, as indicated earlier. Humic acid, obtained by extraction with pyrophosphate solutions, usually contains a higher ash content than that extracted by the NaOH method. According to Stevenson (1994), this is due to higher metal contents in the pyrophosphate extract, and a subsequent treatment for reducing the ash content often results in a significant loss in humic acid.

As with the case of fulvic acid, humic acid will not melt upon heating. However, it will decompose upon heating in an air or nitrogen atmosphere or even in vacuum. During heating in vacuum, it is reported to form a resin-like substance accompanied by the release of water (Orlov, 1985). Similarly as with fulvic acid, the decomposition of humic acid takes place without burning, but through a gradual process of charring. However, it may start to burn at 150°C to 240°C in the presence of oxidizing agents (Hoffman and Schnitzer, 1968). Irreversible changes start to appear clearly on heating at 105°C, and Orlov recommends avoiding the use of elevated temperatures in the study of humic acids. Drying of humic acid is suggested as a better method to be conducted at 50°C in vacuum over P₂O₅.

6.6.2.1 *Humic Acid Fractions*

6.6.2.1.1 *Hymatomelanic Acid*

This is the humic acid fraction soluble in ethanol. Of all the other fractions, it is perhaps the best-known fraction of humic acid. It is believed to contain polysaccharide components in ester linkages (Tan and Clark, 1968; Tan, 1975).

6.6.2.1.2 *Alpha (α) Humic Acid*

This is the remaining humic acid fraction after separation of the hymatomelanic fraction; hence, it is the fraction insoluble in ethanol. In the past, this ethanol-insoluble fraction was called humic acid, and the original humic acid before ethanol treatment was given the name α -humic acid (Russell and Russell, 1950). However, as explained in a preceding section, it is more logical to assign the name α -humic acid to the remaining portion of the humic substance after separation from the hymatomelanic part and retain the name humic acid for the original substance.

6.6.2.1.3 *Beta (β) Humic Acid*

This is a fraction isolated by Waksman (1936) from a fulvic acid solution. As indicated earlier, by adjusting the pH of the acidic solution of fulvic acid to 4.8,

a precipitate is produced which is assigned the name β -humic acid. Although many seem to agree with this, the question was raised earlier of how fulvic acid can yield a humic acid fraction. To some scientists, the substance is comparable to Al-humate. However, the present author is of the opinion that the name Al-fulvate is perhaps more suitable. Although fulvic acid is, by definition, soluble in acidic solutions, in the form of an Al-fulvate it can precipitate at pH 4.8.

6.6.2.1.4 Brown Humic Acid

This is the humic acid fraction soluble in neutral salt (0.1 M NaCl or KCl) solutions. It is highly dispersable and acidic in reaction. The brown color is reported to fade after long standing. This fraction is believed to be prevalent in the humic acids of peat, brown coal, and alfisols (Stevenson, 1994).

6.6.2.1.5 Gray Humic Acid

This is the humic acid fraction insoluble in neutral salt (0.1 M NaCl or KCl) solutions. It is less acidic (milder) than the brown humic acid fraction, and the gray color is relatively stable upon long standing. Although this gray humic fraction is reported to disperse in water, it is also noticed that it can be easily coagulated. Stevenson (1994) believes that gray humic acid is a typical fraction of humic acids in chernozems and rendzinas, soils classified today as mollisols.

6.6.2.1.6 High Molecular Weight Fraction (M.W. >30,000)

This is the humic acid fraction that is eluted first as a result of separation by Sephadex G-50 gel filtration. It makes up approximately 50% of the total humic acid compound (Tan and Giddens, 1972).

6.6.2.1.7 Low Molecular Weight Fraction (M.W. = 30,000–15,000)

This is the humic acid fraction that is eluted last from a Sephadex G-50 column. It amounts to approximately 50% of the total humic acid compound.

6.6.2.1.8 Fractions A, B, R_p, and P

Kumada (1987) claims to be able to distinguish four types of humic acids, types A, B, R_p, and P. From his descriptions, types A and B are in fact humic acid fractions, fractionated using 1 N MgSO₄ according to the old method of Simon and Speicherman (1938). The fraction insoluble in MgSO₄ is called type A, whereas the soluble fraction is called type B. This is then the reason why these types of humic acids are placed in this section about fractions. Whether the remaining R_p and P humic acids can also be considered as

types is not clear. Humic acid types R_p and P are distinguished by Kumada by differences in their absorption spectra and $\Delta \log k$ values. Humic acid type P is reported to originate from humic acids extracted from spodosols, but can be divided further by gel chromatography into a P_b (brown) and P_g (green) fraction. As discussed earlier, the existence of a green humic acid fraction was later invalidated by Kumada.

6.6.3 Humin

This is a type of humic substance that is insoluble in alkaline and acidic solvents. It is the substance that remains behind after extraction of the soil with dilute alkali for humic and fulvic acids. Because of its insolubility and consequent difficulties in extraction and isolation, humin is the compound that has attracted the least research attention, although its presence has been known since the early days of humic acid science. The opinion exists that humin is a mixture of highly condensed humic acids strongly bonded to clay minerals, fungal melanins, and paraffinic compounds (Stevenson, 1994; Kononova, 1966; Somani and Saxena, 1982). Hatcher et al. (1985) disagree about humin being a clay–humic acid complex, whereas others believe that humin has a composition close to that of humic acids, although perhaps slightly less aromatic than the latter (Schnitzer and Khan, 1972). This is assumed to be related to aliphatic polysaccharide components in the humin molecule, which cause humin to be insoluble in alkaline solvents. The higher polysaccharide content in humin is sometimes used to distinguish it from humic acid, although emphasis has recently been placed on the paraffinic constituents for differentiating it from humic acid (Hatcher et al., 1985). The paraffinic substances are believed to be derived from nonvascular plants contributing to the formation of peat.

6.6.3.1 Types of Humin

Hatcher et al. (1985) make a distinction between humin in aerobic and humin in anaerobic (peat) soils. Both types or groups of humin appear to be similar in composition, and differ only in degree and rate of decomposition of the polysaccharide and paraffinic constituents in the humin molecule. These components are better preserved in anaerobic than in aerobic environments. The authors above assume that in the formation of humin, some kind of selective preservation of the organic components occurs. Polysaccharides or carbohydrates in general are known to decompose first

from soils. In addition, Hatcher and coworkers believe that humic acids are the degradation products of humin rather than precursors or are compounds genetically unrelated to humin. They consider humic acids in ancient sediments nothing more than degradation products of humin. In addition, the authors assume that with geologic age, humin can be transformed under anaerobic conditions into coal or kerogen without passing first into a humic acid form.

6.6.3.1.1 Aerobically Derived Humin

This is the type of humin present in terrestrial soil where aerobic conditions prevail. It is believed to be different from humic acid because of its higher aliphatic character due to the presence of high amounts of polysaccharide and paraffinic constituents in its molecule. In the older literature, humin is reported to contain 40% carbohydrates (Russell and Russell, 1950).

6.6.3.1.2 Anaerobically Derived Humin

This is the type of humin in peat and other aquatic environments. A distinction is made by Hatcher et al. (1985) between humin from peat and marine deposits. The authors believe that *peat humin* is composed of lignin, polysaccharides, and paraffinic substances. Some are even claimed to be a two-component system, composed of 60% paraffinic and 40% lignoid substances, because of selective degradation processes. Carbohydrates are considered to be the major constituents of humin at the peat surface, but decrease in concentration with depth due to breakdown by decomposition. Paraffinic substances are said to be selectively preserved in the humin with depth in the peat deposit. In contrast to peat humin, the authors above claim that *marine humin*, from algal-sapropel deposits, is composed almost entirely of polysaccharides and paraffinic substances. However, a similar selective decomposition process is reported to occur with this type of humin. The authors above note that the polysaccharide content in the humin also decreases with depth in the sapropel deposit, whereas the paraffinic components increase due to selective preservation processes.

6.6.3.2 Extraction Procedures

Humin may be extracted by boiling the soil residue, following extraction of humic and fulvic acids, in a 0.5 N NaOH solution. Repeated treatments with a mixture of HCl and HF of the residue are suggested before alkaline extraction. Since humin is believed to exist as a humic-clay complex, the treatment

above is considered required to break the bond with the clay and silica materials, enhancing extraction of the compound (Stevenson, 1994). A more rigorous method is refluxing the soil residue with 6 N HCl (Passer, 1957), whereas Hatcher et al. (1985) recommend pretreatment of the soil residue successively with benzene/ethanol to remove lipids and 1 N HCl, before extraction of humin. The use of a concentrated HCl + HF mixture (1:1 v/v) is even suggested for marine sediments in order to remove by hydrolysis the strongly bonded polysaccharide and protein components. The authors above believe that such a drastic HCl + HF treatment has no effect on the extracted humin, but others are of the opinion that even the use of HCl alone may alter the extracted humin. The acidified sample is then extracted for humin by washing with 0.1 N HCl. This procedure is supposed to yield a hydrogen-saturated humin.

Recently, a new method was introduced for isolation of humin by partitioning a 0.5 M NaOH soil (or peat) extract acidified to pH 1.0 between methyl isobutyl ketone (MIBK) and water (Rice and MacCarthy, 1989). The material suspended in the MIBK phase is, according to the authors above, humin.

6.6.3.3 Humin Fractions

6.6.3.3.1 Inherited Humin

This is a humin fraction separated by ultrasonic treatment (Almendros and Gonzalez-Vile, 1987). It is assumed to be a mixture of degradation products of lignin polymers and subcellular components of plants.

6.6.3.3.2 Humin Fractions of Rice and MacCarthy (1989)

The humin in the MIBK phase above is, in the authors' term, *disaggregated* by adding water. After vigorous shaking, three fractions are produced: (1) a lipid fraction in the MIBK phase, referred to as bitumen by Stevenson (1994); (2) a humic acid-like fraction in the alkaline solution phase; and (3) an insoluble nonhumic fraction, usually settling at the bottom of the flask.

Not much is known yet on the physico-chemical and biological characteristics of humin and its several fractions. The electrochemical properties, including cation exchange and chelation capacity, remain a mystery, providing new frontiers for detailed investigations. The significance and impact of the results of such research on the soil ecosystem and the environment will no doubt also be very prominent, because humin is an important part of the organic cycle. The results of limited investigations showing total acidity

values of humin, ranging from 326 to 636 cmol/kg (Rice and MacCarthy, 1989), are signs for the presence of enormous chemical activities, although less than those of fulvic and humic acid. However, more research is needed to advance or support this knowledge. What is found most important today is the contribution of humin to the carbon cycle and its possible conversion with geologic age into fossil fuel.

Chapter 7

Chemical Composition of Humic Matter

7.1 Elemental Composition

7.1.1 Weight Percentage

As indicated in Chapter 1, the elemental composition of humic matter is a big issue among scientists, with one group being very critical about the presence of an elemental composition and the other group proclaiming the existence of an elemental composition characterizing humic matter. A number of scientists in the first group fail to see the significance of an elemental composition, indicating that extraction procedures may have effected changes in the elemental composition of humic substances. However, Steelink (1985) declares that this issue is not clear yet and needs to be resolved by more detailed research. Except for a generous number of criticisms, no further information of interest is available from this group, and as stated by Ziechmann (1994), elemental composition is a dead issue among these scientists. In contrast, a lot of information have been supplied by the second group. Many of them have analyzed the C, H, O, N, and S contents of humic and fulvic acids extracted from a variety of soils in tropical and temperate regions. These are considered the major elements in humic matter, and a summary of the data is given as examples in Table 7.1. The lignite samples listed in the table were from the deposits in North Dakota, and the data for the lignite–humic and fulvic acids are the average figures of data reported by Mathur and Parnham (1985), Steelink (1985), Tan et al. (1991), Lobartini et al. (1992), and from unpublished data of the present author. The elemental

Table 7.1 Summary of Elemental Composition of Humic Acids (HA) and Fulvic Acids (FA) Extracted from Tropical and Temperate Region Soils and Miscellaneous Environments

| | C | H | O | N | S | C/N |
|-------------------------------|------|-----|------|-----|------|------|
| | % | | | | | |
| Tropical Region Soils | | | | | | |
| HA-alfisols | 52.3 | 5.2 | 37.2 | 3.6 | n.d. | 14.5 |
| HA-andosols | 58.5 | 3.9 | 34.8 | 3.8 | 0.3 | 15.4 |
| HA-oxisols | 54.5 | 4.4 | 38.0 | 3.1 | n.d. | 17.5 |
| FA-andosols | 48.9 | 4.3 | 44.5 | 2.3 | n.d. | 21.3 |
| Temperate Region Soils | | | | | | |
| HA-alfisols | 56.8 | 5.0 | 33.6 | 4.6 | n.d. | 12.3 |
| HA-aridisols (solonetz) | 54.5 | 4.1 | 36.4 | 5.0 | n.d. | 10.9 |
| HA-histosols (peat-bogs) | 58.7 | 5.0 | 32.9 | 3.4 | n.d. | 17.3 |
| HA-inceptisols | 51.4 | 5.4 | 39.1 | 4.1 | n.d. | 12.5 |
| HA-mollisols | 53.7 | 4.3 | 36.3 | 3.7 | n.d. | 14.5 |
| HA-spodosols | 53.1 | 4.9 | 39.2 | 3.5 | 0.4 | 15.2 |
| HA-ultisols | 50.5 | 5.2 | 40.0 | 3.9 | 0.5 | 12.9 |
| FA-inceptisols | 47.9 | 5.2 | 44.3 | 2.6 | n.d. | 18.4 |
| FA-mollisols | 41.6 | 4.0 | 51.9 | 1.1 | n.d. | 37.8 |
| FA-spodosols | 50.6 | 4.0 | 44.1 | 1.8 | 0.3 | 28.1 |
| FA-ultisols | 45.1 | 4.5 | 48.0 | 2.2 | 0.3 | 20.5 |
| Geologic Deposits | | | | | | |
| Lignite | 52.6 | 2.8 | 31.8 | 2.0 | 0.7 | 26.3 |
| HA-lignite | 57.4 | 3.9 | 36.8 | 1.2 | 0.8 | 47.8 |
| FA-lignite | 46.4 | 4.4 | 45.8 | 1.5 | 0.6 | 30.9 |
| Rivers and Swamps | | | | | | |
| HA-aquatic | 49.6 | 4.8 | 43.5 | 2.1 | n.d. | 23.6 |
| FA-aquatic | 50.8 | 4.3 | 43.1 | 1.6 | n.d. | 37.8 |

(continued)

Table 7.1 (Continued) Summary of Elemental Composition of Humic Acids (HA) and Fulvic Acids (FA) Extracted from Tropical and Temperate Region Soils and Miscellaneous Environments^a

| | C | H | O | N | S | C/N |
|---------------------------|------|-----|------|-----|------|------|
| | % | | | | | |
| Merck Chemical Co. | | | | | | |
| HA-artificial | 59.8 | 4.8 | 33.6 | 1.8 | n.d. | 33.2 |
| “Reference” humic acid | 56.2 | 4.7 | 35.5 | 3.2 | 0.8 | 17.6 |
| Peat | 55.5 | 5.4 | 33.1 | 3.0 | n.d. | 18.5 |
| Plant residue | 49.6 | 6.3 | 41.6 | 2.5 | n.d. | 19.8 |

Source: Lobartini, J. C. et al., *Comm. Soil Sci. Plant Anal.*, 28, 787–796, 1997; Lobartini, J. C. et al., *Geoderma*, 49, 241, 1991; Lobartini, J. C. et al., *Sci. Total Environ.*, 113, 1–15, 1992; Tan, K. H. et al., *Comm. Soil Sci. Plant Anal.*, 22: 861–877, 1991; Kumada, K. *Chemistry of Soil Organic Matter*. Japan Sci. Soc. Press, Tokyo, 1987; Thurman, E. M., and R. L. Malcolm, *Environ. Sci. Technol.*, 15, 463–466, 1981; Orlov, D. S. *Humus Acids of Soils*. Moscow University Press. Translated from Russian (K. H. Tan, ed.) Amerind Publ., New Delhi, India, 1985; Steelink, C.: Implications of elemental characteristics of humic substances. In: *Humic Substances in Soil, Sediment, and Water. Geochemistry, Isolation, and Characterization*. 1985. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Schnitzer, M. et al., *Soil Sci. Soc. Am. J.*, 55, 745–750, 1991; Schnitzer, M., and P. Mathur, *Soil Sci. Soc. Amer. J.*, 42, 591–595, 1978; Schnitzer, M., and S. U. Khan. *Humic Substances in the Environment*. Marcel Dekker, New York, 1972; Cranwell, P. A., and R. D. Hayworth. The chemical nature of humic acids. In: *Humic Substances. Their Structure and Function in the Biosphere*, D. Povoledo, and H. L. Golterman (eds.). Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Centre for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 13–18, 1975; Tan, K. H., and J. Van Schuylenborgh, *Neth. J. Agric. Sci.*, 7, 1–22, 1959.

Note: The figures are averages of weight percentages from multiple analyses of various sources. n.d. = not determined.

composition of aquatic humic and fulvic acids are also the average figures of data supplied by Lobartini et al. (1991), Thurman and Malcolm (1981), and from unpublished data of the present author. The black water or humic water—listed under rivers and swamps as aquatic—samples for extraction of these humic substances are from the Okefenokee Swamp, Satilla River, Ochopee River, and Suwannee River in the southeastern United States.

Historical data reported as early as 150 years ago by Mulder and other pioneers in humic acid science, for %C (56%–62%), %H (2.0%–5.5%), and %N (2%–8%), are remarkably within the range of those listed in Table 7.1. However, Orlov (1985) cautions their use for comparison, because they are in the higher ranges, due perhaps to the habit in the old days of drying humic acids at 140°C or higher, and the use of different values in atomic weights.

Both the old and especially the modern data show the elemental composition to be within relatively fixed limits, meaning that it does not exhibit an erratic, random, or a very wide range of variation, as would have been expected with fake materials or artifacts. The differences noticed may be due to differences in origin or to the types of humic substances. Orlov (1985) believes that variation in the elemental composition is affected by (1) variability in soils, (2) variability of humic substances in time and space, (3) different isolation techniques, and (4) errors in sampling and analyses. Nevertheless, the general composition of humic matter, as listed in Table 7.1, still shows a close relation with that of the plant material from which it has been derived. The observed divergence from the plant residue is apparently the result of the humification process and other soil factors. The composition is also in the range of that of humic acid, listed as a “reference” in the table. This particular sample is considered the ideal example of humic acid in soils by Steelink (1985) and Schnitzer and Mathur (1978).

7.1.1.1 Carbon Content of Humic Substances

With few exceptions, the carbon content of humic acids is similar to slightly higher than that in plant residue. On the other hand, fulvic acids exhibit carbon contents almost similar to slightly lower than that in plant residue. The differences in carbon contents between the two types of humic substances are in agreement with data supplied by Steelink (1985), showing carbon contents of 53.8% to 58.7% in humic acids and 40.7% to 50.65% in fulvic acids. This may indicate that fixation of carbon or *carbon sequestration* takes place during the synthesis of humic matter and goes up slightly with an increased rate of humification. The decomposition of organic residue is characterized generally by a loss of C in the form of CO₂; however, when humification occurs, some of the C will be incorporated into humus and humic matter. It is estimated that approximately one-third of the C from plant residues is retained in the soil, and stabilization of nonaromatic C is expected to take place through microbial transformation into aromatic substances used in the

synthesis of humic matter. By comparison with the elemental composition of the plant residue listed at the bottom of the table, the carbon contents of humic and fulvic acids indicate that losses of C from decomposition of plant residue should have been minimal. Most of the liberated carbon is apparently retained in the soil in the form of humic matter, since both humic and fulvic acids exhibit C contents in the range of that in plant residues.

7.1.1.2 Hydrogen, Oxygen, and Nitrogen Content

The hydrogen content of humic and fulvic acids does not differ much from each other. The data for both of the humic substances are also in the range of the average hydrogen values of 3.2%–6.2% and 3.8%–7.0% in humic and fulvic acids, respectively, as reported by Steelink (1985). The oxygen contents listed in Table 7.1 for fulvic acids tend to be slightly higher than the average values of 39.7% to 49.8% as reported by Steelink; however, the big difference is in the nitrogen contents. The data in Table 7.1 display nitrogen contents of 2.6% to 5.05% for soil humic acids, which are considerably higher than Steelink's average values of 0.8% to 4.3% N, whereas those of fulvic acids, ranging from 1.1% to 2.6%, tend to be lower than the average values of 0.9% to 3.3% as reported by Steelink. It is conspicuous that soil humic acid is substantially higher in N than soil fulvic acid, which is not apparent from Steelink's average ranges. The exceptions to the above are the geologic and aquatic humic substances, which are characterized by low N contents. The differences in N content are also not too obvious between aquatic humic and fulvic acids.

7.1.2 C/N Ratio of Humic Substances

The carbon-to-nitrogen ratio is often considered an index of decomposition of crop residues. Its value varies from 13 to 20 in legume crops, to 40 in cornstalks, or to 80 in straw of cereal crops, and has been reported to be as high as 500 to 800 in sawdust (Brady, 1990; Miller and Gardiner, 1998). The decomposition of plant residues results in some of the carbon and nitrogen being lost, a process considered part of the carbon and nitrogen cycles. However, some of the C and a considerable amount of the N liberated are incorporated into microbial cells or fixed in substances used for the formation of humic matter. These processes lead to decreasing the C/N ratios, eventually reaching relatively constant values in soils. In most soils, the C/N ratio falls within narrow limits to about 10 to 15, when decomposition is

virtually “completed,” meaning organic matter decomposition is in equilibrium with the synthesis and accumulation of new organic materials.

This decrease in C/N ratio, reaching a constant value, is now extended to also indicate the degree or rate of the humification process. As indicated above, part of the liberated carbon and a substantial amount of the organic nitrogen are “sequestered” by the humic molecule. Hence, it is generally believed that the C/N ratio will also decrease with increased rate or degree of humification, and C/N ratios between 10 and 15 are often considered to be characteristic of well-developed humic acids. The data listed in Table 7.1 show some support for the opinion above by displaying C/N ratios of soil humic acids between 12.3 and 17.3. Higher C/N ratios are exhibited by aquatic and geologic humic acids, which is perhaps caused by disturbance in the humification process due to the different environments in aquatic or geologic systems from that in the soil ecosystem, e.g., lower N contents and reduced condition in aquatic systems.

Of interest to note is the high C/N ratios of soil fulvic acids, ranging from 18.4 to 37.8, in comparison with those of humic acids. As discussed above, the differences in carbon contents between fulvic and humic acids were rather small and would not have caused the values of C/N to differ that much. In contrast, the nitrogen content is approximately two to three times higher in humic acids than in fulvic acids, which may indicate that fixation of N increases with increased humification from fulvic to humic acid. Increasing amounts of nitrogenous compounds are apparently being sequestered in the process of the synthesis of a humic acid molecule. This raises the possibility that the polymerization or condensation theory of formation of humic acids is not quite correct. Polymerization only of fulvic acids is not adequate, since it would not be able to increase the N content so that it causes the C/N ratio of the polymerized product (humic acids) to decrease that much. The polymerization of 2, 10, and 100 mol (or even higher) of fulvic acids will change neither the composition nor the C/N ratio. Therefore, in addition to polymerization, other reactions, such as interaction, adsorption, chelation of nitrogenous substances, and today's self-assembling processes, are apparently also involved. To raise the nitrogen content in humic acid, it is necessary to also invoke these reactions for the inclusion of the needed nitrogen sources in the humic acid molecule.

The differences in N content between fulvic and humic acids can perhaps be explained partly by invoking the biopolymer degradation theory. Since in this theory, humic acid is formed first, it is more likely that the degradation process yields humic acids with less N than the plant residue,

or maximal with N contents similar to that of the parent material. This will not explain the N contents of humic acids, which are higher than that in the plant residues. However, by further degradation of humic acids into fulvic acids, the possibility arises that in this process, large amounts of the nitrogenous constituents have been broken down and removed from the humic acid molecule. The losses of N in comparison with C must be substantial, causing the N contents to be very low and the C/N ratio to become high in fulvic acids.

7.1.3 Atomic Percentage

A number of scientists believe that elemental composition based on weight percentages cannot be used to explain the molecular structure of humic substances. For the purpose of studying and devising structural formulas for humic substances, they suggest the use of *atomic percentages*. This method of expressing the elemental composition of humic matter is especially popular in eastern European countries (Cięslewicz et al., 1997; Dębska, 1997).

Orlov (1985) is of the opinion that atomic percentages give a better picture than weight percentages on the composition of these substances and on the role they play in molecular structure. He has used them for the determination of molecular weights and formulas, and in distinguishing two groups of humic acids, one with an atomic percentage of 40% to 42% C, and the other with an atomic percentage of 37% to 38%. Steelink (1985) also believes that atomic percentages and atomic ratios are useful as guides in the identification of different types of humic acids, and for drafting structural formulas of the humic substances. Hence, the data in Table 7.1 are converted by the present author into atomic percentages and the results are listed in Table 7.2.

In comparison with weight percentages, the variation in elemental composition expressed in terms of atomic percentages is also relatively small. The humic and fulvic acids from soils and aquatic environments exhibit C, H, O, and N atomic percentages within relatively narrow fixed limits, also providing strong credentials for the presence of real natural compounds, instead of fake or operational substances.

The data suggest that for every C atom, there is at least one H atom. It is also more evident now that the composition of humic substances contains approximately one atom of O to two atoms of C. Again, one can notice that, in general, the N content is larger for humic acid than for fulvic acid. The nitrogen atomic percentages are approximately twice that large in humic acids than in fulvic acids of terrestrial soils.

Table 7.2 Summary of Elemental Composition of Humic Acids (HA) and Fulvic Acids (FA) Extracted from Tropical and Temperate Region Soils and Miscellaneous Environments in Atomic Percentages

| | C | H | O | N | ω |
|-------------------------------|---------|------|------|-----|----------|
| | Atomic% | | | | |
| Tropical Region Soils | | | | | |
| HA-alfisols | 35.9 | 42.8 | 19.2 | 2.1 | 0.053 |
| HA-andosols | 43.4 | 34.8 | 19.4 | 2.4 | 0.258 |
| HA-oxisols | 39.4 | 38.1 | 20.6 | 1.9 | 0.223 |
| FA-andosols | 36.0 | 38.0 | 24.6 | 1.4 | 0.428 |
| Temperate Region Soils | | | | | |
| HA-alfisols | 38.9 | 41.1 | 17.3 | 2.7 | 0.041 |
| HA-aridisols (solonetz) | 40.3 | 36.4 | 20.2 | 3.1 | 0.330 |
| HA-histosols (peat-bogs) | 40.1 | 41.0 | 16.9 | 2.0 | 0.080 |
| HA-inceptisols | 34.5 | 43.5 | 19.7 | 2.4 | 0.090 |
| HA-mollisols | 39.6 | 38.0 | 20.1 | 2.3 | 0.230 |
| HA-spodosols | 36.8 | 40.7 | 20.4 | 2.1 | 0.174 |
| HA-ultisols | 34.5 | 42.7 | 20.5 | 2.3 | 0.151 |
| FA-inceptisols | 32.9 | 42.8 | 22.8 | 1.5 | 0.222 |
| FA-mollisols | 32.1 | 37.1 | 30.1 | 0.7 | 0.785 |
| FA-spodosols | 38.0 | 36.0 | 24.8 | 1.2 | 0.453 |
| FA-ultisols | 30.9 | 37.4 | 30.8 | 0.9 | 0.871 |
| Geologic Deposits | | | | | |
| Lignite | 47.1 | 30.1 | 21.3 | 1.5 | 0.361 |
| HA-lignite | 43.2 | 35.2 | 20.8 | 0.8 | 0.204 |
| FA-lignite | 34.4 | 39.1 | 25.5 | 1.0 | 0.433 |
| Rivers and Swamps | | | | | |
| HA-aquatic | 35.0 | 40.7 | 23.0 | 1.3 | 0.263 |
| FA-aquatic | 37.3 | 37.9 | 23.8 | 1.0 | 0.340 |

(continued)

Table 7.2 (Continued) Summary of Elemental Composition of Humic Acids (HA) and Fulvic Acids (FA) Extracted from Tropical and Temperate Region Soils and Miscellaneous Environments in Atomic Percentages

| | C | H | O | N | ω |
|---------------------------|---------|------|------|-----------|----------|
| | Atomic% | | | | |
| Merck Chemical Co. | | | | | |
| HA-artificial | 41.5 | 40.0 | 17.5 | 1.0–0.048 | |
| “Reference” humic acid | 39.6 | 39.7 | 18.8 | 1.9 | 0.091 |
| Peat | 37.6 | 43.9 | 16.8 | 1.7–0.138 | |
| Plant residue | 29.4 | 44.7 | 24.6 | 1.3 | 0.286 |

7.1.4 Internal Oxidation (ω) of Humic Substances

The parameter in Table 7.2, designated by the symbol ω , refers to the internal oxidation value of humic substances. This is considered an important value, especially in Europe, for studying diagenetic changes of humic substances.

7.1.4.1 Degree of Oxidation Theory of Orlov (1985)

A number of scientists believe that diagenetic changes of humic acids are closely related to degradation and oxidation reactions. This hypothesis, as discussed by Orlov (1985), starts with the degradation of plant residue to form a humic acid-like substance. The degradation process involves the loss of CH_3 groups. The transformation of the humic substance above into a humic acid characteristic, for example, in mollisols, is postulated to take place by a continuation of the degradation process, involving now also partial oxidation and further losses of CH_3 groups. An increase or decrease in the number and length of aliphatic chains in the humic molecule is considered to be inherent to this basic process of addition or loss of especially terminal (CH_3) - or (CH_2) - groups. The resulting difference between the numbers of oxygen and hydrogen atoms is used as a measure of the degree of the oxidation processes. This difference can be calculated by several different, although related, methods. The simplest is the method using a revised formula below, adapted from Orlov (1985):

$$\Delta = 2\text{O} - \text{H} \quad (7.1)$$

in which Δ = difference between numbers of oxygen and hydrogen atoms, O = number of oxygen atoms, and H = number of hydrogen atoms. For a water molecule (H_2O), a fundamental basis of this hypothesis, the difference equals zero:

$$\Delta = 2O - H = (2 \times 1) - (2 \times 1) = 0 \quad (7.2)$$

To compare the degree of oxidation in different organic substances, Orlov (1985) proposes to calculate the difference on the basis of 1 g atom of carbon or per unit amount of carbon atoms:

$$\omega = \frac{2O - H}{C} \quad (7.3)$$

in which ω = the difference between numbers of O and H atoms per 1 g atom of C, and C = number of carbon atoms.

The value of ω is negative with substances in the reduced state, meaning in substances with excess H atoms. The value of ω is positive with substances in the oxidized state, or substances containing excess oxygens. The value is considered to fluctuate between a minimum of -4 and a maximum of +4, corresponding to the formation of CH_4 and CO_2 , respectively. Methane, CH_4 , is considered a C compound at the highest reduced state, whereas carbon dioxide, CO_2 , is assumed to be a C compound at the highest oxidation state. Examples of calculations are given below as illustrations:

$$CH_4: \quad \omega = (0 - 4)/1 = -4 \quad (7.4)$$

$$CO_2: \quad \omega = (4 - 0)/1 = +4 \quad (7.5)$$

Applying formula 7.3, Orlov argues that the degree of oxidation in the formation of a carbohydrate, with a general formula of $C_x(H_2O)_y$, must equal zero:

$$\omega = \frac{2O - H}{C} = \frac{2y - 2y}{x} = 0 \quad (7.6)$$

7.1.4.2 Internal Oxidation Hypothesis of Dębska, Gonet, and Cięslewicz

Orlov's hypothesis on the degree of oxidation, as discussed above, is considered by some scientists in Europe inadequate to explain the formation of humic substances. It is an established fact that, in addition to C, H, and O,

humic substances also contain nitrogen as an additional major constituent. Hence, another hypothetical model has recently been proposed to include the significance of N in the formation and diagenetic changes of humic substances. The degree of the oxidation process, used above as the basis for reflecting formation and diagenetic changes of humic substances, is now called *internal oxidation*. The relationship, by which the degree of internal oxidation can be calculated, is formulated as follows (Dębska et al., 2012, 2009; Dębska, 1997; Cięslewicz et al., 1997):

$$\omega = \frac{(2O + 3N) - H}{C} \quad (7.7)$$

Formula 7.7 was allegedly suggested by Zdanow in 1965, as cited by Dębska et al. (2012). The rationale of adding 3N is not clear and can only be guessed. Perhaps it is based on the formation of NH₃ as the most reduced N compound, since the value of $\omega = 0$ for the formation of NH₃ gas, as shown below:

$$\text{NH}_3: \quad \omega = (2 \times 0) + (3 \times 1) - (3 \times 1) = 0 \quad (7.8)$$

Using Equation 7.7, the values for ω have been calculated by the present author for the humic substances, using the atomic percentages in discussion as listed in Table 7.2. As can be noticed from the data, most of the values for ω are positive in sign, indicating a high degree of internal oxidation. Only HA-histosol, extracted from peat bog samples, HA-artificial, and peat have ω -values that are negative, indicating a low degree of internal oxidation. In Orlov's terms, this means that humic acids extracted from soils have excess oxygens; in other words, they are compounds in an oxidized state. On the other hand, humic acid from peat, peat itself, and humic acid artificially prepared by the Merck Chemical Company have excess hydrogen atoms, or are compounds that are in a reduced state. The above lends support to the more recent findings by Cięslewicz et al. (1997), who conclude from their research that positive values for ω are exhibited by humic acids extracted from well-drained soils, underscoring the aerobic environment prevailing in soils affecting the oxidation of humic acids. In contrast, the negative values for ω are noticed by Cięslewicz et al. (2008, 1997) for humic acids extracted from lagoon sediments, indicating anaerobic conditions in the transformation of humic acids. The positive values

of ω for the aquatic humic substances (Table 7.2) perhaps indicate that they are allochthonous, hence have been formed in aerobic terrestrial ecosystems before being transported into aquatic environments by erosion, percolation, and/or leaching.

7.1.4.3 Significance of ω in Humification Processes

The issue of internal oxidation values of humic substances seems to be very complex, if not confusing, and a simple definition based on a rationale and that is easy to understand is also apparently needed. As it stands now, it is considered an important parameter in humic acid chemistry in Europe. Humification is allegedly closely related to the degree of internal oxidation. Dębska and coworkers suggest that its value can be used to indicate the stage of the humification process. The more advanced the rate and degree of the humification process—or in their words the more mature the humic acids—the higher will be the ω values. The authors above believe that humification proceeds with the humic molecules exhibiting an increase in O₂ and a decrease in H₂ contents, and hence suggest that the smaller the H/C ratios the higher will be the degree of humification (Dębska et al., 2009; Szombathova, 2004). The data in Table 7.3 list internal oxidation (ω) values from their investigations of humic acids extracted from chernozemic soils and luvisols, soils that are called mollisols and alfisols, respectively, in U.S.

Table 7.3 Effect of Cultivation on Internal Oxidation Values (ω) and Elemental Composition (in Atomic%) of Humic Acids from Chernozems (Mollisols) and Luvisols (Alfisols)

| Sample Origin | C | H | N | O | H/C | ω |
|------------------------|-------|-------|------|-------|------|----------|
| Chernozem under forest | 33.75 | 43.18 | 2.79 | 20.29 | 1.28 | 0.171 |
| Cultivated chernozem | 38.27 | 39.42 | 2.68 | 19.64 | 1.03 | 0.206 |
| Luvisol under forest | 34.47 | 42.37 | 2.46 | 20.70 | 1.23 | 0.186 |
| Cultivated luvisol | 34.40 | 41.96 | 2.37 | 21.27 | 1.22 | 0.223 |

Source: Dębska B. et al., *Polish J. Soil Sci.*, 42, 131–138, 2009.

Soil Taxonomy (Soil Survey Staff, 1990). As can be noticed, the atomic percentages of oxygen do not make any difference; however, it is the decrease in H content, which decreases the H/C ratio, that is claimed to be the main reason for the ω values to increase in humic acids from cultivated soils.

Cultivation then allegedly causes humification to proceed to advanced levels. Since Dębska and coworkers stated only the differences in ω values and did not provide any explanations for the discrepancies, one has to assume that humification is not “mature” yet in soils under forest vegetation and is still in process. The author can only speculate that such a condition is perhaps due to a continuous supply of fresh forest litter to the soils under forest.

This seems to be supported by the other data, where Dębska and coworkers indicate that applications of organic residues in the form of farmyard manure have decreased the internal oxidation value of humic acid (Dębska et al., 2012; Szombathova et al., 2004).

7.1.5 Atomic Ratios

For the purpose of creating structural formulas or formula weights, the ratios of the atomic percentages H/C, O/H, O/C, and N/C are found to be helpful. In addition, Steelink (1985) thinks that they can also be used for the identification of types of humic substances. Hence, the atomic ratios are calculated by the present author from the data listed in Table 7.2, and the results are presented in Table 7.4.

7.1.5.1 Significance of Atomic Ratios in Identification of Humic from Nonhumic Substances

The atomic ratios of H/C of the humic substances (Table 7.4), ranging from 0.90 to 1.35, are in agreement with those reported by Orlov (1985) and Steelink (1985). As indicated earlier by Orlov (1985), the figures suggest that for each carbon atom, there is indeed one hydrogen atom in the humic molecule. The atomic H/C ratios of aquatic humic matter are also within the range of the limits stated above. However, the present data do not support the observation of Ishiwatari (1985) for higher H/C ratios for humic substances in lake sediments than those in soils. The present maximum of 1.35 for humic substances conforms fairly well with the contention of Chen et al. (1977) that H/C ratios >1.3 indicate the presence of nonhumic compounds. As can be noticed in Table 7.4, the H/C atomic ratio of plant residue equals 1.5.

Table 7.4 Atomic Ratios of Major Elements in Humic and Fulvic Acids and Miscellaneous Materials

| | <i>H/C</i> | <i>O/H</i> | <i>O/C</i> | <i>N/C</i> |
|--------------------------------|------------|------------|------------|------------|
| Tropical Region Soils | | | | |
| HA-alfisols | 1.192 | 0.449 | 0.539 | 0.058 |
| HA-andosols | 0.802 | 0.559 | 0.447 | 0.055 |
| HA-oxisols | 0.967 | 0.540 | 0.523 | 0.048 |
| FA-andosols | 1.055 | 0.647 | 0.683 | 0.039 |
| Temperate Regions Soils | | | | |
| HA-alfisols | 1.121 | 0.392 | 0.439 | 0.071 |
| HA-aridisols (solonetz) | 0.903 | 0.055 | 0.501 | 0.077 |
| HA-histosols (peat-bogs) | 1.022 | 0.412 | 0.421 | 0.050 |
| HA-inceptisols | 1.352 | 0.417 | 0.564 | 0.069 |
| HA-mollisols | 0.960 | 0.529 | 0.508 | 0.058 |
| HA-spodosols | 1.105 | 0.501 | 0.554 | 0.057 |
| HA-ultisols | 1.237 | 0.480 | 0.594 | 0.067 |
| FA-inceptisols | 1.301 | 0.533 | 0.693 | 0.046 |
| FA-mollisols | 1.155 | 0.811 | 0.938 | 0.022 |
| FA-spodosols | 0.947 | 0.689 | 0.653 | 0.032 |
| FA-Ultisols | 1.210 | 0.824 | 0.997 | 0.029 |
| Geologic Deposits | | | | |
| Lignite | 0.639 | 0.708 | 0.452 | 0.032 |
| HA-lignite | 0.815 | 0.591 | 0.481 | 0.019 |
| FA-lignite | 1.136 | 0.652 | 0.741 | 0.029 |
| Rivers and Swamps | | | | |
| HA-aquatic | 1.162 | 0.565 | 0.657 | 0.037 |
| FA-aquatic | 1.016 | 0.628 | 0.638 | 0.027 |

(continued)

Table 7.4 (Continued) Atomic Ratios of Major Elements in Humic and Fulvic Acids and Miscellaneous Materials

| | H/C | O/H | O/C | N/C |
|---------------------------|-------|-------|-------|-------|
| Merck Chemical Co. | | | | |
| HA-artificial | 0.964 | 0.438 | 0.422 | 0.024 |
| "Reference" humic acid | 1.00 | 0.474 | 0.475 | 0.048 |
| Peat | 1.167 | 0.383 | 0.447 | 0.045 |
| Plant residue | 1.520 | 0.550 | 0.837 | 0.044 |

7.1.5.2 Use of Atomic Ratios in Differentiating Humic from Fulvic Acids

The atomic ratios of O/C (Table 7.4) show values that are in agreement with those reported by Orlov (1985) and Steelink (1985). As is the case with the other authors, the present data also clearly separate soil humic acids from soil fulvic acids. Humic acids exhibit O/C values ranging from 0.421 to 0.657, whereas fulvic acids are characterized by O/C values between 0.653 and 0.997. This is in agreement with Steelink (1985), who also reports lower O/C atomic ratios in humic acids (averaging 0.5) than in fulvic acids (averaging 0.7). Steelink is of the opinion that the O/C ratio is the best parameter for differentiating between types of humic compounds.

The O/C ratios of aquatic and lignite humic matter also fall within the range of those of soil humic matter. These values are noted to be lower in the humic acids than in the fulvic acid compounds extracted from lignite. However, the O/C values are not much different between aquatic humic and fulvic acids, contradicting Steelink's (1985) report for higher O/C ratios in aquatic fulvic than in humic acid.

Although Steelink (1985) is of the opinion that the O/C ratio was the best, the present data in Table 7.4 indicate that the N/C ratios are equally adequate for differentiating between humic and fulvic acids. As can be noticed, soil humic acids exhibit N/C ratios ranging from 0.048 to 0.077 as opposed to those of soil fulvic acids with N/C ratios in the range of only 0.022 to 0.046. Extremely low N/C ratios are noticed for aquatic and lignite-derived humic and fulvic acids.

The observations above suggest that, in general, the humic molecule contains more carbon atoms than oxygen atoms, which is in close agreement with the assumption made by Orlov (1985). The humic acid molecule has at least two carbon atoms for one oxygen atom. The O/H ratios indicate the presence of one oxygen atom to two hydrogen atoms, a composition similar to that of a water molecule, and according to Orlov (1985) also similar to carbohydrates. The higher O/C ratios reaching values of 0.99 in fulvic acids suggest a composition made up of one carbon to one oxygen atom. Such a ratio is common for carbohydrate molecules. For example, the carbohydrate $C_6H_{12}O_6$ has six carbon atoms to six oxygen atoms per molecule, giving an O/C ratio = 1.0. This observation increases the credibility of opinions that fulvic acids are polysaccharidic in nature. Steelink (1985) and Ishiwatari (1975) believe that the carbohydrate content increases during the formation of fulvic acid. Such an assumption is perhaps correct when considered from the standpoint of the polymerization concept, where fulvic acid is the starting point in the humification process. During formation and polymerization of humic precursors into fulvic acids, more polysaccharides or carbohydrates are incorporated than phenolic or quinonic substances. However, from the viewpoint of the biopolymer degradation concept, it is unlikely that during the degradation process into fulvic acid, more carbohydrates are being sequestered. By adding more carbohydrates into its molecule, the humic substance to be formed will not be reduced in size and is expected to be similar in molecular size as humic acid. Hence, the proposal is now presented that during the transformation into fulvic acid, more of the aromatic substances are broken down and released by comparison with the polysaccharide constituents. The latter increases in amount because of the larger losses of phenolic and quinonic constituents. Consequently, the fulvic acid formed becomes increasingly more polysaccharidic in nature.

Finally, the values of N/C ratios of soil humic acids suggest, on average, a composition of 0.06–0.05 nitrogen atoms to 1 carbon atom. This means that approximately 1 nitrogen atom is present against 16–20 carbon atoms in a humic acid molecule. In fulvic acid, the molecule is expected to contain at least 1 nitrogen atom against 50 atoms of carbon.

7.2 Group Composition

In addition to elemental composition, group composition can also be used in the characterization of humic substances. Instead of being composed of

elements or atoms, this composition is made up of chemical units of atoms or compounds; hence, the name *group composition* is used here by the present author. It plays an important role in determining the chemistry and structural properties of humic substances. Composing a structural formula and the study on chemical behavior of humic substances would be incomplete without the knowledge of group composition. The chemical reactions characteristic of humic substances are attributed, for the most part, to the existence of a particular group composition. It is not enough to know how many atoms are present in a humic molecule; however, it is, in addition, necessary to learn about the units created by the atoms. Although the issue of group composition is discussed in the literature many times, often in considerable length, not much concrete data are available. At present, it is known that it can be distinguished into (1) a functional group composition and (2) a group-compound composition. For readers interested in the methods of determination of both the functional groups and group composition of humic matter, reference is made to Tan (2000), Stevenson (1994), and Purdue (1985).

7.2.1 Functional Group Composition

The functional groups are composed of a set of active chemical groups that gives the humic substances their unique chemical behavior. Interaction reactions characteristic of humic substances, e.g., complex formation, chelation, and ion or metal bridging, are attributed to their presence in the humic molecule. These processes will be discussed in more detail in Chapter 9.

These groups are sometimes referred to by different names. Stevenson (1994) suggests naming them *oxygen-containing functional groups*, although some of the groups do not contain oxygen at all. In contrast, Purdue (1985) prefers to call them *acidic functional groups*, which also limits the groups to units that behave as acidic only. The major types of functional groups are in the form of COOH, phenolic-OH, alcoholic-OH, and carbonyl groups. These units indeed contain oxygen atoms. In addition to the above, amino groups are also important functional groups; however, these are neither of the oxygen-containing nor of the acidic types.

7.2.1.1 Carboxyl Groups

The carboxyl or COOH groups give the humic molecule its acidic characteristic. Their presence is the reason why humic substances exhibit charge

properties and have the capacity to adsorb and exchange cations. As indicated in Chapter 9 and as can be noticed in Figure 9.1, these carboxyl groups will dissociate their H atoms at pH 3.0. Such behavior—donating protons—fits the concept of a Brønsted–Lowry acid. However, Purdue (1985) considers the acidity of humic substances to be attributable to their proton-binding capacity. Elaborate statistical models have been presented by the author for describing proton binding that can be used as a basic foundation for the acidity of humic substances, before finally admitting that none of them were satisfactory. The latter is due to the extreme complexity of the distribution of acidic functional groups in the humic molecule.

Not much information is available in the literature on carboxyl content of humic substances. Most of the published material is often on the analytical procedures of determination (Stevenson, 1994; Purdue, 1985), and only some authors discuss the amounts present in humic matter. The most common method used for determination is the Ca-acetate titration procedure (Tan, 1995); however, other chemical methods are also available. Carbon-13 nuclear magnetic resonance (^{13}C -NMR) spectroscopy (Schnitzer and Preston, 1986) and x-ray diffraction analysis (Schnitzer et al., 1991) have also proven to be useful in the determination of COOH content in humic substances. However, the figures reported show large variations due to differences in methods of determination. Although many think that these data are in disagreement and should be interpreted with caution, the present author notices that data obtained by the same analytical procedure agree fairly satisfactorily, with variations occurring only within very narrow limits. A summary of the average values of such data is provided in Table 7.5. As can be noticed, the carboxyl content in soil humic acids is in the range of 2.4 to 5.4 meq/g, which is substantially lower than those in fulvic acids with COOH values of 8.5 meq/g. These values, obtained by the Ca-acetate titration method, are in the range for COOH content summarized by Stevenson (1994) from data reported by Schnitzer (1977) at the International Atomic Energy Agency meetings in Vienna. However, the COOH values for humic acids are rather low when compared with the average values of 6.4% to 9.0% obtained by ^{13}C -NMR analysis (Schnitzer and Preston, 1986). Higher values for COOH content have even been reported by Hatcher et al. (1981b), who obtained by NMR analysis concentrations of 10 to 11 meq/g in humic acids from inceptisols. In the opinion of Schnitzer and Preston (1986), this discrepancy is attributed to the inclusion of amides and esters in the analysis by NMR spectroscopy.

Table 7.5 Summary of COOH, Phenolic-OH Contents, and Total Acidity in Soil, Geologic, and Aquatic Humic Matter

| | <i>Carboxyl</i> | <i>Phenolic-Hydroxyl</i> | <i>Total Acidity</i> |
|--------------------------|-----------------|--------------------------|----------------------|
| | <i>meq/g</i> | | |
| Soil Humic Matter | | | |
| HA-alfisols | 3.9 | 2.8 | 6.8 |
| HA-inceptisols | 2.4 | 3.6 | 6.0 |
| HA-spodosols | 5.4 | 3.5 | 8.9 |
| HA-ultisols | 3.7 | 4.4 | 8.1 |
| FA-spodosols | 8.5 | 3.8 | 12.3 |
| FA-ultisols | 8.5 | 1.5 | 10.0 |
| Geologic Deposit | | | |
| HA-lignite | 5.2 | 0.9 | 6.1 |
| FA-lignite | 6.6 | 4.9 | 11.5 |
| Rivers and Swamps | | | |
| HA-aquatic | 3.4 | 2.5 | 5.9 |
| FA-aquatic | 3.9 | 2.3 | 6.2 |

Source: Schnitzer, M., and S. U. Khan. *Humic Substances in the Environment*. Marcel Dekker, New York, 1972; Schnitzer, M. Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones. Proc. Symposium Soil Organic Matter Studies, Braunschweig. Int. Atomic Energy Agency, Vienna, pp. 117–131, 1977; Tan, K. H. et al., *Comm. Soil Sci. Plant Nutr.*, 21, 1999–2016, 1990; Tan, K. H. et al., *Comm. Soil Sci. Plant Anal.*, 22, 861–877, 1991; Lobartini, J. C. et al., *Geoderma*, 49, 241–254, 1991; Lobartini, J. C. et al., *Sci. Total Environ.*, 113, 1–15, 1992.

On the other hand, lack of reactivity and steric hindrance are the reasons cited by the authors for the low percentages in the titration procedures. Analytical constraints on acidic functional group determination are apparently not limited to the above. Purdue (1985) indicates that the amount of oxidic groups behaving as acids is limited by the oxygen content in a humic substance. On the basis of oxygen content alone, Purdue argues that humic acid with an oxygen content of 48% will contain 15 mmol/g of COOH or 30 mmol/g in the form of phenolic-OH or other oxy-type acidic groups.

Unsaturation of COOH and phenolic groups is another possibility for the variations in the results of determination of COOH contents.

Stevenson (1994) agrees that fulvic acids are higher in COOH content than humic acids. According to this author, the amount of COOH groups is inversely related to molecular weights, and fulvic acids are known to be lower in molecular weights than humic acids. He is of the opinion that the oxygen-containing functional groups are the highest in fulvic acids than any of the other naturally occurring organic polymers. The COOH concentration is considered to decrease upon *carbonization*, a theory advanced by Kumada (1987) for the formation and transformation of humic matter. Stevenson (1994) argues that all humic and fulvic acids surviving biological attack are eventually diagenetically transformed into kerogen or coal. If humic and fulvic acids are affected by the *coalification* process, the first to disappear are the COOH groups, followed by methoxyl, OCH₃, and C=O groups.

7.2.1.2 Hydroxyl Groups

Humic substances contain a variety of hydroxyl groups; however, for characterization of humic acids, generally three major types of OH groups are distinguished, as follows:

1. *Total hydroxyls*. They are the OH groups associated with all functional groups, such as phenols, alcohols, and hydroquinones. However, often the term “total hydroxyls” refers only to the sum of phenolic- and alcoholic-OH groups. Total OH is usually measured by acetylation.
2. *Phenolic-hydroxyls*. These are OH groups attached to phenolic (aromatic) structures. Currently, there is no wet chemical method for the determination of these groups. The amount is usually determined by difference as follows:

$$\text{meq phenolic-OH} = \text{meq total acidity} - \text{meq COOH} \quad (7.9)$$

3. *Alcoholic-hydroxyls*. They are OH groups associated with alcoholic groups or nonaromatic carbons. The amount can also be determined by difference only:

$$\text{meq alcoholic-OH} = \text{meq total OH} - \text{meq phenolic-OH} \quad (7.10)$$

The reactivity of alcoholic-OH groups is usually considered lower than that of phenolic-OH groups; hence, the latter are assumed to be the most

important in humic acid reactions. These phenolic-OH groups are another reason for giving the humic molecule its charge characteristic and unique chemical behavior. In basic chemistry, hydroxyls react as bases. However, phenolic-hydroxyls react as weak acids since they dissociate their protons at pH 9.0 (see Figure 9.1). Because of this dissociation, Stevenson (1994) prefers to call them acidic-OH rather than phenolic-OH groups, whereas Purdue (1985) prefers to use the name weakly acidic groups.

When electrically charged, the phenolic-OH groups together with the COOH groups provide the capacity for interaction reactions. Complex reactions, chelation, and water and metal bridging are made possible as shown in Figure 9.2.

As can be noticed from Table 7.5, the phenolic-OH content in soil humic acid is in the range of 1.5 to 4.4 meq/g, which agrees with values reported by Schnitzer (1977). Not many other authors have studied concentrations of phenolic-OH groups in humic matter since no direct wet chemical methods are available. As indicated above, its measurement can be conducted only indirectly. The amount of aromatic carbon in the humic molecule is said to also limit the phenolic-OH concentration in humic substances (Purdue, 1985), which no doubt adds to the difficulties in the determination.

7.2.1.3 Total Acidity

This is not a functional group, but it is a very important characteristic of humic substances closely related to the functional groups. It is commonly used as a measure for the cation exchange capacity of humic substances. Although it can be determined directly by titration, the common method is to calculate it by summation as follows:

$$\text{meq total acidity} = \text{meq COOH} + \text{meq phenolic-OH} \quad (7.11)$$

However, the use of different analytical procedures in the determination of total acidity or carboxyl groups has been noted to often yield different results, as explained earlier. This is also shown by Felbeck (1965), who demonstrates that total acidity measurements by the Ba(OH)_2 , KOH, NaOH, or Ba-acetate procedures generally yield different results. Nevertheless, by limiting collection and comparison of data within similar methods, the present author notes that the variation in values occurs only within narrow limits. The average figures in Table 7.5 for total acidity determined by the summation method vary only from 6.0 to 8.9 meq/g for soil humic

acids, and from 10 to 12.3 meq/g for fulvic acids. These values are in agreement with those reported by Stevenson (1994), Hatcher et al. (1981), and Schnitzer (1977). The big difference in total acidity between humic and fulvic acid is also supported by the authors above. Stevenson indicates that fulvic acids are unmistakably substantially higher in total acidity than humic acids, which is due to their higher COOH content. Extremely high values of 14 meq/g are in Stevenson's opinion caused by analytical errors, such as failure to remove inorganic acids from the samples before titration.

In summary, the conclusion can be made that soil humic acid is characterized by a carboxyl content ranging from 2.4 to 3.9 meq/g, and a phenolic-OH content of 2.8 to 4.4 meq/g. These differences between the carboxyl and phenolic-OH content are so small that, in essence, one can conclude that humic acid has equal amounts of COOH and phenolic-OH groups. On the other hand, fulvic acid appears to contain almost twice the amount of carboxyl groups as humic acids. The amount of COOH in fulvic acid averaging 8.5 meq/g is also substantially higher than the phenolic-OH content, which is 2.6 meq/g on average. Such differences in functional group composition result in remarkably large differences in total acidity values between humic and fulvic acids, as noticed from the data in Table 7.5. Humic acids from lignite and aquatic humic and fulvic acids exhibit COOH and phenolic-OH contents in the range of soil humic matter discussed above. However, no marked differences have been observed between COOH and phenolic-OH contents in aquatic humic matter.

7.2.2 Group-Compound Composition

This composition is made up of well-defined compounds, such as carbohydrates, aromatic, and carboxyl group compounds. Such information proves to be valuable in the identification of types of humic substances. It is usually determined by cross-polarization magic angle spinning (CP-MAS) ^{13}C -NMR spectroscopy (Tan, 2000; Hatcher et al., 1980). Humic matter samples in the solid and liquid state can be analyzed, whereas soil samples in the undisturbed state can also be used, provided the samples contain sufficient amounts of organic matter (Tan et al., 1992). The results of NMR analysis yield humic acid spectra that can be divided into several regions:

1. Aliphatic C region at 0 to 105 ppm chemical shift
2. Polysaccharide subregion that can often be distinguished from 65 to 105 ppm

3. Aromatic C region at 105 to 165 ppm chemical shift
4. Carboxyl C region at 165 to 185 ppm chemical shift

The intensity of the signals (peaks) for aliphatic, aromatic, and carboxyl C may vary depending on the origin (differences in soils) and type of humic matter analyzed. These peak resolutions form the basis for calculating the group composition percentages by integration of the spectra. An example of such data on group composition is provided in Table 7.6. Generally, fulvic acids exhibit spectra dominated by strong signals for aliphatic C, which yield by integration high percentages of aliphatic carbon. The signals for aromatic C are often manifested as very weak peaks, which then translate into low percentages of aromatic carbon in fulvic acids. On the other hand, humic acids show spectra with weaker signals in the aliphatic regions but with very strong signals in the aromatic regions. This gives, by integration, low percentages of aliphatic carbon and high percentages of aromatic carbon, respectively. The data also show that the group composition of aquatic fulvic acid from the black water streams closely resembles that of soil fulvic acids, providing evidence that the aquatic fulvic acids from the streams of the southeastern United States are allochthonous in nature (Tan et al., 1990).

Table 7.6 Average Group-Compound Composition of Humic and Fulvic Acids Extracted from Soils, from Black Water Streams in Southeastern United States, and from Lignite

| | Aliphatic C | Aromatic C | Carboxyl C | Aromaticity | Aliphaticity |
|-----------------------------------|-------------|------------|------------|-------------|--------------|
| % | | | | | |
| Soils | | | | | |
| Humic acid | 37.3 | 51.6 | 11.1 | 58.0 | 42.0 |
| Fulvic acid | 61.6 | 20.7 | 17.7 | 25.2 | 74.8 |
| Humic Water or Black Water | | | | | |
| Fulvic acid | 61.6 | 22.3 | 16.1 | 26.6 | 73.4 |
| Geologic Deposit | | | | | |
| Lignite | 63.3 | 30.6 | 6.1 | 32.6 | 67.4 |
| Humic acid | 27.2 | 63.8 | 9.0 | 70.1 | 29.9 |
| Fulvic acid | 45.7 | 34.9 | 19.4 | 43.4 | 56.6 |

Source: Tan, K. H. *Environmental Soil Science*. Second Edition. Marcel Dekker, New York, 2000; Tan, K. H. et al., *Comm. Soil Sci. Plant Anal.*, 23, 1513–1532, 1992.

7.2.2.1 Aromaticity

This is a measure of the relative amount of phenolic or aromatic structures in a humic molecule. In discussing the issue of *aromaticity*, it should be realized that the aspect of *aliphaticity* is included inherently. Aromaticity is a function of the group-compound percentages, providing information on how much of the aromatic compounds are present relative to the aliphatic substances in the molecular structure of humic matter. Hence, once one is determined, the other is automatically known. Data on aromaticity and/or aliphaticity are not only of importance for assessing the origin, stability, and chemical behavior of humic substances, as indicated by Schnitzer et al. (1991), but they can also be used for distinguishing between types of humic substances.

The conventional method of determination of aromaticity is to analyze first by ^{13}C -NMR spectroscopy the percentages of aromatic, aliphatic, and carboxyl carbon, as explained above. However, Schnitzer et al. (1991) have reported that these percentages can also be measured by x-ray diffraction analysis. This is rather surprising, since this method is normally used to analyze crystalline compounds in soils and fails to yield satisfactory results with amorphous substances. More details about this issue will be given in Chapter 8. Nevertheless, the value for aromaticity can then be calculated using the weight percentages obtained by applying either one of the following formulas:

$$\text{Aromaticity} = \frac{\text{Aromatic C\%}}{\text{Total C\%}} \times 100\% \quad (7.12)$$

$$\text{Aromaticity} = \frac{\text{Aromatic C\%}}{\text{Aliphatic C\%} + \text{Aromatic C\%}} \times 100\% \quad (7.13)$$

Schnitzer et al. (1991) indicate that ^{13}C -NMR analysis determines both aromatic + phenolic C; hence, aromatic + phenolic C should be used instead of aromatic C% alone, as shown in Equation 7.13. The contention that NMR spectroscopy analyzes both aromatic and phenolic C is indeed quite correct, but the term *phenolic* is also referring to a similar aromatic structure; hence, using aromatic + phenolic C is, in essence, redundant. Equation 7.12 will yield slightly lower aromaticity percentages than Equation 7.13, but the differences are relatively small. The percentage of aromaticity for soil humic acid in Table 7.6 equaling 58.0% is determined by Equation 7.13. Using Equation 7.12, it amounts to only 51.6%, and differences from 0% to 8% have also been detected in humic acids by Schnitzer et al (1991).

7.2.2.2 Aliphatic Humic Substances

The data in Table 7.6 clearly show sharp differences in aromaticity between humic and fulvic acids. Humic acids appear to be twice as aromatic as fulvic acids, a fact supported by the data of Schnitzer and his coworkers (1991), revealing average percentages for aromaticity in humic acids as high as 71.8% and 64.8%, as compared with 36.0% and 31.5% in fulvic acids, determined by x-ray diffraction and NMR analyses, respectively. Higher aromaticity in humic acids is considered by some to be the result of the humification process. During humification, it is believed that the amount of aromatic and alkyl carbons increases, whereas the O-alkyl carbon content decreases. This idea is based on the opinion that the carbohydrates (O-alkyl carbons) are broken down by the soil microbes, resulting in relatively increasing the aromatic and alkyl fractions (Chefetz et al., 2002). The issue of the aromatic and aliphatic nature of humic substances has been examined in some detail in Chapter 5, Section 5.3.6.1, where it has been addressed that polysaccharides are important components of fulvic acid molecules. Hymatomelanic acids are also noted to contain polysaccharides in their core structures. With the rise of the science in aquatic organic matter, this idea on the aliphatic nature of humic substances seems to increase substantially in importance. It is obvious now that true aquatic humic compounds are derived from indigenous plants that do not contain aromatic substances. The data listed in Table 5.1 indicate that the aquatic sources, phytoplankton, zooplankton, and diatoms, for the formation of autochthonous humic matter do not contain lignin, the sources for the aromatics. The information above reinforces the significance of the presence of “aliphatic” humic matter.

7.3 Theory of Minimum Formula Weights

Formula weights are molecular formulas derived from elemental compositions calculated in atomic percentages as shown in Table 7.2. The empirical formulas are normally used for expressing molecular weights in chemistry and, therefore, their value in representing molecular masses depends highly on the reliability, accuracy, and precision of elemental analyses. The topic of minimum weight formulas is discussed in this section—rather than in the molecular weight section of Chapter 8—because of its close dependence and ease of referencing the data on C, H, O, and N, and their atomic ratios listed in Tables 7.2 and 7.4.

Formula weights have attracted considerable attention since the beginning of humic acid science, acquiring over the years the notoriety of molecular weights in defining humic acids. In previous publications, Piccolo (2002) Piccolo et al. 2003 and Tan (2003a, 2011a, b) have addressed in some detail the alleged failure in finding a magic number for molecular weight as one of the major reasons to question the existence in nature of humic substances. At one time, they were even called operational or fake compounds and were it not for their ubiquitous presence in the earth ecosystems, humic acid science may perhaps have taken a wrong turn. As discussed in Chapter 3, Section 3.4.1, and Chapter 4, Section 4.1, it was perhaps Berzelius in 1839, followed by Mulder in 1840, who presented the first formulas for their ulmic, crenic, and apocrenic acids. Using today's chemical notations, the compounds above were allegedly assigned the chemical formulas $C_{20}H_{14}O_6$, $C_{12}H_{12}O_8$, and $C_{24}H_{12}O_{12}$, respectively. This was the starting-point for debate, which turned into a full-blown controversy over the existence of molecular weight formulas, especially when more formulas have been presented over the years as humic acid chemistry advances into a modern science. Unfortunately, the differences in opinion have polarized the scientific community, resulting in a division into two groups, with one group firmly believing in and the other highly critical about the existence of formula weights for humic substances. Regardless of the convictions on formula weights from the group of non-believers, several methods have been reported for the construction of such formulas by the group of believers. Steelink (1985) has also stated unequivocally "yes" to the question whether a reasonable structural formula can be created for humic acids. The most modern methods are reported by Schnitzer (1994) and Schulten (1994, 1996), who are using sophisticated methods, such as pyrolysis–field ionization mass spectrometry (Py–FIMS) and Curie-point pyrolysis–gas chromatography (Py–GC), to come up with formulas and graphical models of humic structures. Not only are these methods mind boggling, but the analytical instruments are also extremely expensive and out of reach for many similarly competent scientists. Less complex but also qualified and easy to follow are the methods proposed by Steelink (1985) and Orlov (1985), which is presented below by the present author more coherently from the bits and pieces gathered from the respective articles and book.

7.3.1 Calculation of Formula Weights

Both Steelink (1985) and Orlov (1985) have used elemental compositions expressed in atomic percentages and atomic ratios for the calculation of

formula weights of humic substances. In contrast to Steelink (1985), who considers only C, H, and O, Orlov (1985) includes N in the formulation. As indicated in Chapter 3, Section 3.4.1, Orlov also assumes the humic substances to be characterized by a *minimum molecular weight*, defined as the smallest possible formula of humic acids. A combination of Orlov's and Steelink's methods is presented below as an illustration of how to calculate an empirical weight formula for humic acid from the elemental composition and atomic ratios in Tables 7.2 and 7.4, respectively. In addition, similar data of humic acids from Orlov's book (1985, Table 9) are used for comparisons and/or supporting data. It needs to be underscored here that all the data used are not fictional but are data obtained from actual analyses of humic substances extracted from soil samples collected by Tan worldwide and by Orlov in Russia. The average values from Tan's data listed below are used:

| <i>Atomic Ratio</i> | <i>Range (Tan)</i> | <i>Average</i> | <i>Range (Orlov)</i> | <i>Average</i> |
|---------------------|--------------------|----------------|----------------------|----------------|
| H/C | 0.8–1.2 | 1.0 | 0.8–1.2 | 1.0 |
| O/C | 0.4–0.6 | 0.5 | 0.4–0.5 | 0.5 |
| N/C | 0.05–0.07 | 0.06 | 0.04–0.08 | 0.06 |

As can be noticed, the average values of Orlov and Tan for H/C and O/C are similar. They are also not different from those used by Steelink (1985) in his calculations. It could be coincidental, but the stunning similarities from three independent researchers tend to reinforce the idea of the existence of a definite composition, hence supporting the idea of humic acids being discrete or real compounds in nature. Regardless of what the reasons are, the above atomic ratios mean that for every carbon, there are 1 hydrogen, 0.5 oxygen, and 0.06 atoms of nitrogen, resulting in a *working formula* of $C_1H_1O_{0.5}N_{0.06}$, as shown below. By rules of chemistry, formula notations should carry whole numbers, instead of broken numbers, by either adjusting with a factor and/or rounding up to the next higher whole number. Consequently, conforming to Orlov's (1985) suggestion, the smallest broken number in the working formula of 0.06, the subscript for N, has to be adjusted by a factor of $1/0.06 = 16.7$ to equal 1 (one) as shown below

0.06 N: $C_1H_1O_{0.5}N_{0.06}$ (*working formula*)

1N: $C_{16.7}H_{16.7}O_{(16.7 \times 0.5)}N$ or rounded up: $C_{17}H_{17}O_8N$, mol.wt. = 363

2N: $C_{34}H_{34}O_{16}N_2$, mol.wt. = 726

3N: $C_{51}H_{51}O_{24}N_3$, mol.wt. = 1089

| | |
|---|------------------|
| 4N: C ₆₈ H ₆₈ O ₃₂ N ₄ , | mol.wt. = 1452 |
| 5N: C ₈₅ H ₈₅ O ₄₀ N ₅ , | mol.wt. = 1815 |
| 10N: C ₁₇₀ H ₁₇₀ O ₈₀ N ₁₀ , | mol.wt. = 3630 |
| 30N: C ₅₁₀ H ₅₁₀ O ₂₄₀ N ₃₀ , | mol.wt. = 10,690 |

The subscripts of C, H, and O have then to be adjusted accordingly and the broken subscript numbers rounded up, giving the formula C₁₇H₁₇O₈N, as shown above. This is called the *minimum molecular weight* of the humic substance above. As can be noticed, it corresponds to the simplest formula and the real (total) values are then the multiples of this minimum formula weight value, such as exemplified by the formula C₅₁₀H₅₁₀O₂₄₀N₃₀. The latter corresponds to a molecular weight of 10,690, a value close to the lower limits of molecular weights often reported for humic acids (Bogoslovskiy and Levinskiy, 2006; Stevenson, 1998).

7.3.2 Minimum Molecular Weight as a Property of Humic Matter

The idea above has indeed proved very controversial, but may fulfill the need for the existence of a generally accepted molecular weight characterizing humic substances. The use of minimum molecular weights is, in fact, very common in the chemistry of natural biopolymers, such as with carbohydrates that exhibit a minimum molecular weight expressed by the formula C₆H₁₂O₆ or with protein by a minimum weight formula of C₂H₅O₂N (glycine). The total carbohydrate or whole protein molecule is then the multiples of either C₆H₁₂O₆ or C₂H₅O₂N. The method above is much simpler and based on a more solid scientific foundation than that of Schnitzer (1994) and Schulten (1996), who have presented with high-technology procedures a formula of C₃₀₈H₃₂₈O₉₀N₅ for humic acids. It has to be admitted, however, that their goal was to create the “true” formula. The authors also claim their formula above to correspond with an elemental composition of 66.8% C, 6.0% H, 26.0% O, and 1.3% N. Unfortunately, the extremely high %C and very low %O are out of range for values reported for humic substances (Stevenson, 1998; Schnitzer and Khan, 1972). The low value of N also gives a C/N ratio of 66.8/1.3 = 51.4, a value far too high for humic acids, and infringes on the basic concepts of humification. A C/N ratio that high is indicative for the specimen of Schnitzer and Schulten to be more likely raw “nonhumified” organic matter. For comparison, the elemental composition of some of the plant biopolymers is provided in Table 7.7. As can be noticed, the elemental composition of lignin, although not a perfect match, resembles

Table 7.7 Average Elemental Composition of Biopolymers (Wt.%)

| <i>Biopolymer</i> | C | H | O | N | S |
|-------------------|----|-----|------|-----|-----|
| Lignin | 63 | 5.0 | 31.6 | 0.3 | 0.1 |
| Carbohydrates | 44 | 6.0 | 5.0 | — | — |
| Protein | 58 | 7.0 | 22 | 17 | 1.0 |
| Lipid | 76 | 12 | 12 | — | — |

Source: Orem, W. H., and P. G. Hatcher, *Int. J. Coal. Geol.*, 8, 33–54, 1989; Martinelli, G. Petroleum chemistry, Chapter 4. In: *Petroleum Engineering-Upstream*, E. Mesini, and P. Macini (eds.). UNESCO, EOLSS Publishers, Oxford. <http://www.eolss.net>, 2009.

closely that of the alleged humic acid of Schnitzer and Schulten. It is not clear and very confusing why Schnitzer and Schulten did not use the composition of humic acids reported in their same papers for the udic Borolls, haplaquods, or haplaquolls. The data from either of these soils or from their average values are more related to humic acids present in or extracted from soils than those of “proposed” (fake) humic acids. The use of a hypothetical humic acid is unfortunate since it may aggravate the issue of artifacts.

7.3.3 *Formula Weights Including Functional Groups*

If now the humic acid above also contains the following amounts of functional groups (see Table 7.5)

| | <i>Range, meq/g</i> | <i>Average, meq/g</i> |
|--------------------|---------------------|-----------------------|
| COOH groups | 2.4–5.4 | 3.9 |
| Phenolic-OH groups | 2.8–4.4 | 3.6 |

then the humic acid unit capable of containing 3.9 meq COOH and 3.6 meq phenolic-OH groups can be calculated as follows. Since COOH is monovalent, $3.9 \text{ meq} = 3.9 \text{ mmol/g}$ or $3.9 \times 363 \text{ mmol} = 1415 \text{ mmol COOH/mol}$ of humic acid with a formula weight of 363 g. This means that there are 1.4 mol of COOH per $\text{C}_{17}\text{H}_{17}\text{O}_8\text{N}$, or $10 \times 1.4 = 14 \text{ mol COOH groups per } \text{C}_{170}\text{H}_{170}\text{O}_{80}\text{N}_{10}$. This is valid only for the particular humic acid above possessing COOH groups to the amount of 3.9 meq/g. For other humic acid types with different COOH group contents, their formula weights have to be

determined following a similar computational process. However, the lower and/or higher multiple weight formulas of the same sample, which decreases or increases the COOH group contents correspondingly, can be figured out easily as shown below:

| <i>Formula Weight</i> | <i>Mol. Wt.</i> | <i>mol COOH group</i> |
|-------------------------------|-----------------|-----------------------|
| $C_{17}H_{17}O_8N$ | 363 | 1.4 |
| $C_{85}H_{85}O_{40}N_5$ | 1815 | 7.0 |
| $C_{170}H_{170}O_{80}N_{10}$ | 3630 | 14.0 |
| $C_{255}H_{255}O_{120}O_{15}$ | 5445 | 21.0 |

Note that an adjustment factor, e.g., $\times 5$, $\times 10$, $\times 15$, etc., can be used that yields only whole numbers for both the moles of COOH group and the subscripts in the formula weights. Hence, for the particular humic acid sample used above, the adjustment factor of “ $\times 30$ ” then gives $1.4 \times 30 = 42$ mol COOH groups that can be accommodated only by a formula weight of $C_{510}H_{510}O_{240}N_{30}$ with a molecular weight of 10,690.

A similar calculation and reasoning conducted with 3.6 meq/g of the phenolic-OH groups yield 1.306 mol phenolic-OH per mole of humic acid with a formula weight of 363 g. Consequently $10 \times 1.3 = 13$ phenolic-OH groups are present per $C_{170}H_{170}O_{80}N_{10}$. The conclusion can be made that humic acid with a formula weight of $C_{170}H_{170}O_{80}N_{10}$ is capable of accommodating both 14 mol COOH and 13 mol phenolic-OH groups. Because of the uneven figure for the phenolic-OH content, the adjustment factor is then limited to $\times 10$ and higher for yielding whole numbers as formula subscripts.

By including the functional group—COOH and phenolic-OH groups—contents into the procedural concept of minimum formula weights, as discussed above, the theory of minimum formula weight will perhaps increase in scientific value.

7.4 Molecular Structures

In close relation to molecular formulas is the construction of a molecular structure for humic substances. This is a more complex and very difficult problem. Many attempts have been conducted and a monograph was even published by the International Humic Substances Society (IHSS) in 1989 under the title “Humic substances: II. In search for structure” (Hayes et al.,

1989). However, for a detailed and better review of the study of molecular structure and its basic consideration, reference is made to Stevenson (1994) and Orlov (1985).

7.4.1 Issue of Pseudostructures

All of the theories suggested on molecular structures are based on aromatic constituents or phenols obtained by a variety of degradation analyses of humic substances. This is especially exemplified by the IHSS monograph (Hayes et al., 1989), which has a lot of information on degradation techniques but falls short in structural concepts and designs characterizing humic substances. Oxidative degradation, hydrolytic degradation, reductive cleavage, and thermal degradation techniques always yield phenol or benzoic-like units, e.g., methoxybenzaldehydes, methoxybenzenecarboxylic acid, hydroxybenzene, dihydroxybenzene, and the like. Schnitzer and coworkers spent considerable time and effort on these kinds of chemical degradation of humic substances (Schnitzer and Khan, 1972; Schulten and Schnitzer, 1993). Unfortunately, the results showing the production of invariably benzene carboxylic derivatives only, as stated above and discussed in Chapter 5, Section 5.3.2.3, confirm the present author's views on the futility of degradation analyses. These compounds are considered by many to be broken down from large plant biopolymers such as lignin. Recently, a modern and sophisticated method has been used, employing thermochemolysis in the presence of tetramethyl-ammonium hydroxide (TMAH) and Py-GC/mass spectrometry (Py-GC/MS) for the detection of structural units in humic matter (Chefetz et al., 2002). The authors claim that these techniques will provide structural information on building blocks of natural macromolecules in soils without interference from conventional degradation procedures. The TMAH method is said to be highly selective in cleaving ester and certain ester linkages in the humic molecule. The method also avoids decarboxylation and produces the methyl esters of carboxylic acids for easy analysis by GC. Most important, the authors indicate they can detect lignin-derived phenols and benzenes, protein-derived pyridine, and polysaccharide-derived acetic acids and aldehydes by analysis with the Py-GC/MS method. Although all of the above could be true, it is not irrefutably proven yet that the small phenol units have originated from lignin, or pyridine units from protein. It is a matter of believing and trusting only, since as discussed in Chapter 5, phenol or benzene-like compounds are derived from two sources in soils, from lignin and from the synthesis by soil microorganisms.

It is evident by now that regardless of their origin, the building blocks of humic matter detected by all the degradation, thermal, and pyrolysis methods appear to be mostly small phenol monomers. These small units are then rearranged into a structural unit to represent a humic acid structure. Therefore, it is just a structural model showing only molecular components thought to be present in natural humic substances. Critics have suggested naming the derived structural models *pseudostructures* (Essington, 2003), which they define as hypothetical structures containing elemental and chemical components and functional group features consistent with the observed composition, mass, and other properties obtained from pyrolysis, NMR, and mass spectrometry. Real natural humic acid structures, however, have been obtained by various investigators by analyses of whole, nondegraded, or unbroken humic specimens by electron microscopy. Recent examples of such humic acid structures have been reported by the present author in Chapter 3, Figures 3.3 and 3.4, and additional examples will be provided in Chapter 8, Section 8.62, on characterization of humic substances by electron microscopy.

7.4.2 Model Structures Based on the Ligno-Protein Concept

Since a great number of methods and opinions are available in the construction of molecular structures of humic substances, many structural designs have been presented. Some of the structural models are relatively simple, whereas others are very complex and remarkably stunning. Many of the older structures, such as those presented by Fuchs (1930a, b) and Dragunov (1958), will not be shown in this book, since they have been discussed in almost all the other books covering humic acid structures. For these and several other humic acid structures of historical value, reference is made to the books of Stevenson and Orlov, mentioned above. In this section, the various schemes will be sorted according to the different concepts on humification, and only the most pertinent examples will be used as illustration.

7.4.2.1 Lignin Monomer Concept of Flaig

Structural designs of humic acid based on lignin monomer units have been proposed by Flaig (1967) and Steelink (1985). Flaig has studied the disintegration of lignin into the simpler monomers and their further disintegration into smaller phenol units. Most of his ideas on humic acid structures, based on using the phenol units, have been covered in detail in several of the author's publications and also reproduced by other authors (Flaig, 1975; Flaig et al.,

1975, 1959; Stevenson, 1994; Orlov, 1985). However, one aspect that has apparently escaped attention is Flaig's idea of using a lignin monomer for the molecular structure of humic acid. In this case, the author believes that carbohydrates may also play a role; hence, he proposes the incorporation of a carbohydrate molecule in the formulation of a humic structure. A revised version of the structural design is shown in Figure 7.1 with coniferyl alcohol as the main building block. Flaig's structural design is composed only of a phenyl propane unit and a carbohydrate group. However, since protein is now considered an essential component of the humic acid structure, a revision is made by the present author to also include a protein or peptide unit in Flaig's structural model. Without protein, Flaig's structure reflects only a precursor of humic acid. A similar structural design for terrestrial humic acid has been suggested by Tan (2000) in his book entitled *Principles of Soil Chemistry*.

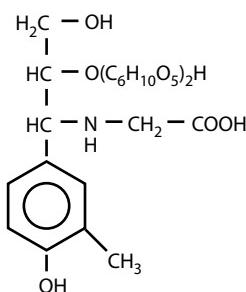
7.4.2.2 Lignin Tetramer Concept of Steelink

A second example of how a lignin monomer can be used in forming a molecular structure of humic acid is proposed by Steelink (1985). This author has calculated, with the atomic ratios of C/H = 1.0 and O/C = 0.5, the smallest formula for humic acid with a phenyl propane backbone to be $C_{18}H_{18}O_9$. To accommodate 1.5 mol of COOH groups per mole of humic acid, Steelink reasons that 2 [$C_{18}H_{18}O_9$] are needed to contain 3 mol of COOH. The formula has then to be changed into $C_{36}H_{36}O_{18}$, which in his opinion translates into a phenyl proprane tetramer. However, one of the three monomers exhibits a terminal COOH group, suggesting it to be a hydroxyphenyl peruvic acid, and not a phenyl propane monomer, as suggested by Steelink in Figure 7.1. Next to this is a quinone, which also does not qualify to be called a phenyl propane. In addition, lack of participation of a peptide unit only reflects the structure of a precursor of humic acid. Steeling's crooked and twisted structural design of the monomers has been corrected in Figure 5.1 to straight lines of -C-C-C-. This is the more common way to represent lignin monomer molecular structures.

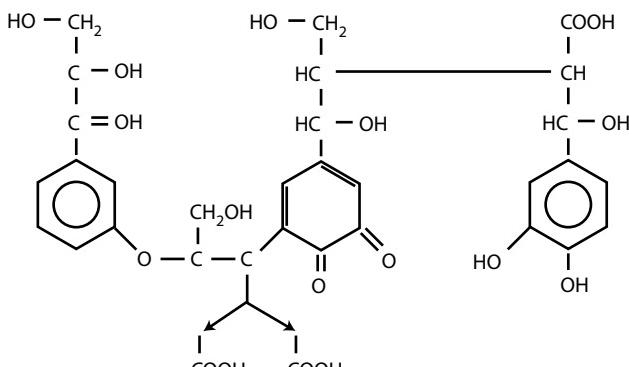
7.4.3 Model Structures Based on the Phenol–Protein Concept

7.4.3.1 Phenol Oligomer Concept of Schulten

Most of the structural designs for humic substances are in this category of the phenol–protein concept, and all of them show complex networks of phenol units linked together by other carbon units. For instance, a tangled



Flaig (1967)



Steelink (1985)

Figure 7.1 Author's version of humic acid structures according to ligno-protein concept. (Adapted from Flaig, W. Chemical composition and physical properties of humic substances. In: *Studies about Humus*. Trans. Int. Symposium "Humus et Planta IV." Czechoslovak Academy of Science, Prague, pp. 81–112, 1967; Steelink, C. Implications of elemental characteristics of humic substances. In: *Humic Substances in Soil, Sediment, and Water. Geochemistry, Isolation, and Characterization*. 1985. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

web of phenolic units covering a whole page has been presented for a structural concept of humic acid by Schulten (1994) and Schnitzer (1994). Schulten (1994) claims to arrive at such design from an extensive literature search, long-term investigations by Py-GC/MS; Py-FIMS; ¹³C-NMR; chemical, oxidative, and reductive degradation; colloid chemical; and electron microscopic studies on soil humic substances. According to Chefetz et al. (2002), these complex instrumentations are only capable of detecting small organic compounds, presumably building blocks of humic substances. Although Schulten's structural concept can be considered as the most advanced design, the complexity and the lack of reasonable explanations, other than the complex analyses, for the

rationale of a network of linkages make most scientists cautious, if not shy away, in using it.

7.4.3.2 Phenol Polymer Concept of Schnitzer and Orlov

Less complex structural designs that are easier to understand have been presented by Schnitzer (1978) and Orlov (1985). Orlov's structural model, presented in Figure 7.2, underscores better than Schnitzer's the concept

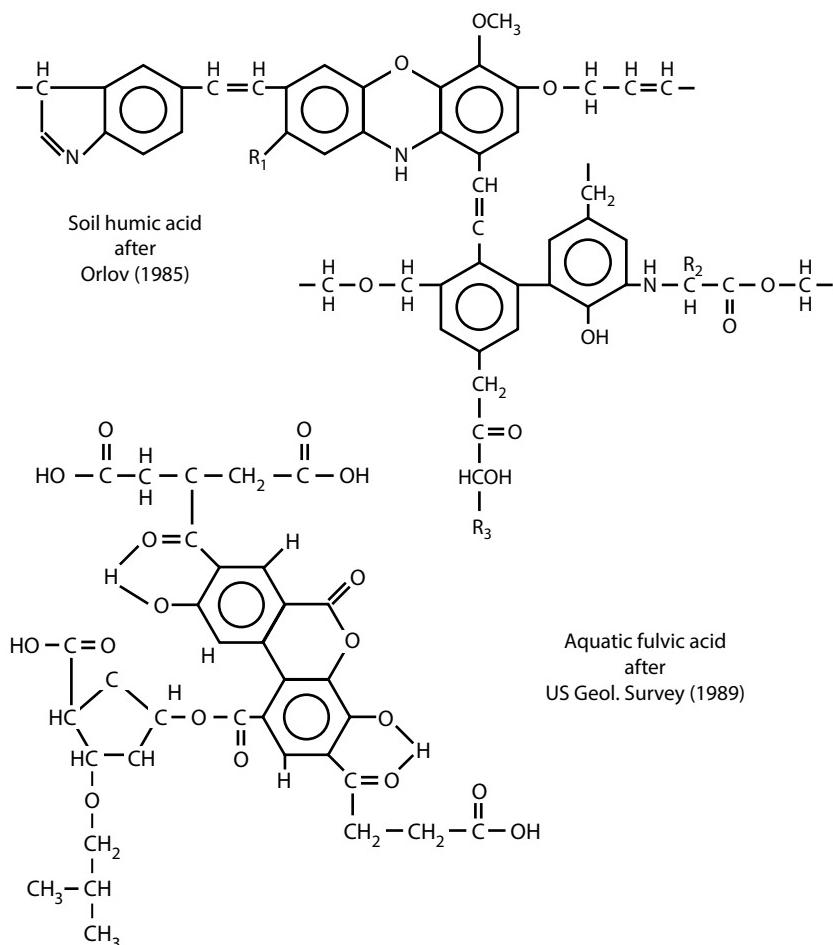


Figure 7.2 Structural models, redesigned by the present author, according to phenol-protein theory. (Adapted from Orlov, D. S. *Humus Acids of Soils*. Moscow University Press. Translated from Russian (K. H. Tan, ed.) Amerind Publ., New Delhi, India, 1985; U.S. Geol. Survey Staff. *Humic substances in the Suwannee River, Georgia. Interactions, properties, and proposed structures. Open File Report 87-557*. U.S. Geol. Survey, Denver, 1989.)

of phenol–protein linkages in the humic acid structure. Structural models of aquatic humic substances proposed by the U.S. Geological Survey Staff (1989) and by Steinberg and Muenster (1985) are also in the same category because of the large number of phenol units making up the structure. The example suggested by the U.S. Geological Survey, also shown in Figure 7.2, is supposed to be a structural model of an aquatic fulvic acid from the Suwannee River in South Georgia, United States. The phenol units making up the structure support the contention made earlier that these fulvic acids are allochthonous in nature. The four terminal carboxyl groups underscore the high acidity of fulvic acid in general, and also support the observation of fulvic acids containing high amounts of COOH groups as discussed above.

7.4.3.3 Phenol Dimer Concept of Stevenson

The simplest design is the structure based on the phenol dimer concept of Stevenson (1994). This author assumes the linkage of two phenol units to which a protein is attached as the basic or smallest unit of a humic molecule (Figure 7.3).

He also believes that the dimer is an excellent unit for explaining the chemical reactions characteristic of humic substances. Other structural designs based on linkages of phenol–protein units are available in the literature, and for interested readers, reference is made to the respective publications listed in the reference lists of Tan (2000), Stevenson (1994), and Orlov (1985).

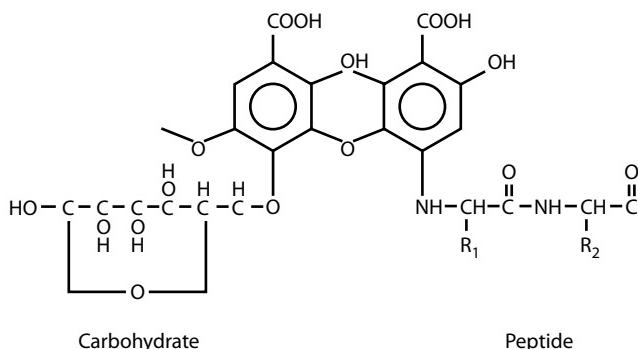


Figure 7.3 Redesigned structural model of humic acid based on the dimer concept of Stevenson (1994). The revision is made by the current author by also including a carbohydrate molecular unit. (From Stevenson, F. J. *Humus Chemistry. Genesis, Composition, Reactions*, Second Edition. John Wiley & Sons, New York, 1994.)

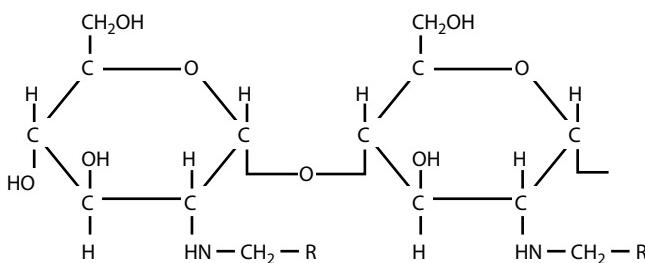


Figure 7.4 Aliphatic structural design showing the linkage of two glucosylamine molecules forming humic acid according to the sugar–amine condensation theory.

7.4.4 Model Structures Based on the Sugar–Amine Condensation Concept

These types of structures are characteristic of autochthonous aquatic humic substances. As explained in Chapter 4 and in Section 7.2.2.2 on aliphaticity of humic substances, the key components for an aquatic humic acid structure are sugar and amine or amino acid, and no lignin derivatives are required. In aquatic environments, plant materials, needed for formation of humic matter, usually do not contain lignin. Especially in marine environments, kelp, seaweed, and plankton, the major sources for the synthesis of marine humic acid, are composed mostly of carbohydrates (see Table 5.1). Therefore, these types of humic acids, if not affected by terrestrial material, exhibit molecular structures composed of carbohydrate and protein units only. As discussed earlier, sugar–amine complexes, called *glucosylamine*, are produced in the Maillard reaction. Condensation or polymerization of the glucosylamine units is considered to form humic substances. A structural model of such a humic acid is illustrated in Figure 7.4, where two or more glucosylamine groups are linked together through oxygen bonds.

7.5 Computer Modeling of Humic Acid Structures

With the recent advances in computer technology, attempts have been made to design computer models of molecular structures for humic acids. Although several methods are available, because of their similarities, only three of the methods will be discussed below. One of the best methods is perhaps that reported by Schulten (1996) in relation to his formulation of the composition of humic acid monomers, trimers, decamers, and pentadecamers. The author claims to have developed a three-dimensional (3D)

structure of a humic acid decamer from a combination of data obtained by wet-chemical, geochemical, biochemical, spectroscopic, and agricultural data and analytical pyrolysis. His wonderful, although bewildering, colored 3D pictures of a humic acid molecule, developed by computer modeling assisted by additional data obtained by Py-FIMS and Curie-point Py-GC/MS, is, of course, quite impressive. A black-and-white reproduction of this structure is provided personally by Schulten and shown in Figure 7.5.

Designing a molecular structure is a very complicated process; hence, to simplify the problem, a free “translation” of Schulten’s discussion is

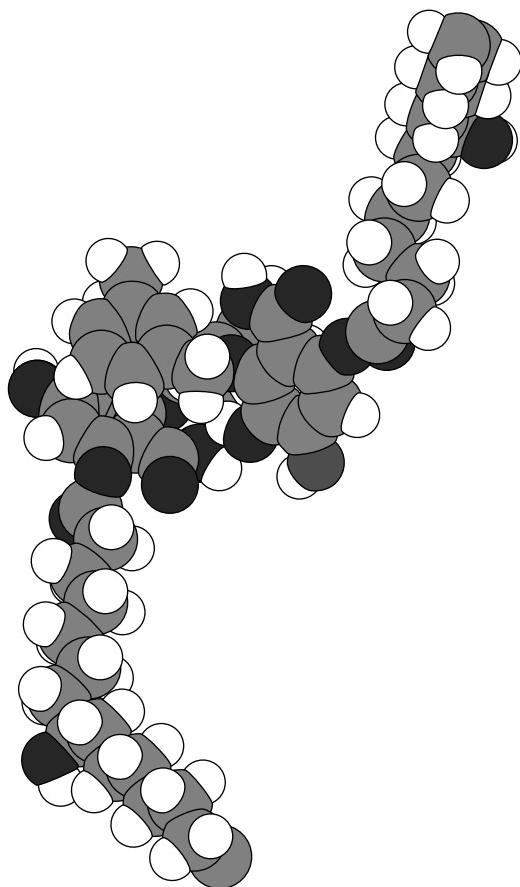


Figure 7.5 Black-and-white three-dimensional computer model of a hypothetical humic acid decamer structure: C = gray, H = white, O = black, and N = not shown. (Provided personally by Schulten and reproduced with permission from Schulten, H.-R. A new approach to the structural analysis of humic substances in water and soils. In: *Humic and Fulvic Acids. Isolation, Structure, and Environmental Role*. ACS Symposium Series 651, pp. 42–56, 1996, and courtesy of American Chemical Society, Washington, DC.)

given below so that it can reach a wider audience of readers. The basis of Schulten's work is a hypothetical humic monomer, characterized by the formula $C_{308}H_{335}O_{90}N_5$, as discussed earlier. He has revised his formula by adding CH_2 to come up with the formula $C_{315}H_{349}O_{90}N_5$, which is converted into the decamer $C_{3150}H_{3482}O_{896}N_{50}$. The latter has an elemental composition of 67.10% C, 6.22% H, 25.43% O, and 1.24% N, which hardly represents the composition generally reported for humic acids by a variety of authors. The ratio of C/N = $67.10/1.24 = 54.1$ indicates more likely the presence of "raw" organic matter, or litter, instead of a humified product as pointed out in Section 7.3.2. None of them also have H/C atomic ratios resembling those discussed earlier (see Table 7.2) for the calculations of minimum molecular weights of humic substances.

In a continuing effort with scientists at the Institute of Soil Science, University Rostock, Germany, a model structure was devised of a humic molecule with another bizarre composition of $C_{6932}H_{7662}O_{1970}N_{110}$, bearing no relationships with the previous ones used above. Although a stunning computer-enhanced 3D structure was published (Schulten and Leinweber, 2000), a copy obtained by Tan—see Figure 3.36 in Tan (2010), obtained with permission of both Dr. Peter Leinweber and Elsevier (the publisher)—shows instead small tangled groups of alleged humic components connected by long aliphatic carbon chains. It raises more questions and controversies about the feasibility of the models representing a humic acid structure, whereas the ensuing personal communications have resulted only in a vague promise by Leinweber for a statement at a later date.

On the other hand, Schulten (2002) justifies his selections above by stating that to simplify the process, it is necessary to use a humic molecule composed of a relatively low number of atoms, giving a corresponding mass below 6000 g/mol. Using HyperChem software, a molecular structure is then drafted by moving two of these molecules so that a phenolic-OH of one molecule is aligned and in close contact with a furan-O of the other molecule. Intermolecular hydrogen bonds and van der Waals forces are assumed to be linking the two molecules together into a dimer. By adding another molecule to the dimer, a trimer can be formed, and so on. In his latest efforts, Schulten (2002, 2001) has used in addition to HyperChem, the ChemPlus version 3.1 program for computing molecular properties, referring apparently to calculation of the amount of energy in the formation of humic acid polymers. An HA dimer is allegedly constructed, similarly to the method discussed above, by moving two geometrically optimized molecules of humic acid monomers along the inertial x-axis toward each other. When the distance between the two monomers is as

close as 0.25 to 0.8 nm, a phenolic-hydroxyl group of one HA is placed in contact with a furan–oxygen of the other HA molecule. An energy gain of 6.37 kJ/mol has been calculated by Schulten for the formation of this HA dimer. An HA trimer can then be formed in a similar way by adding a monomer to the HA dimer or by trapping a monomer in the dimer structure. Tetramers are formed (1) by again trapping a monomer in a trimer HA structure or (2) by linkage of four HA monomers through covalent bonding. *Trapping* is explained as a process of placement of a molecule in a structural void and is considered an important mechanism for adding peptides, sugars, and other xenobiotics to the humic molecule. Linkage is assumed by intramolecular hydrogen and coordination covalent bonds, the latter resulting in complex formation.

The total energy of the covalently bonded HA tetramer is three times higher than that of the “trapped” tetramer and nine times higher than that of the HA monomer. These energy differences may indicate that humic acid polymers are more stable, hence will likely be more resistant to decomposition, than the humic acid monomers.

Schnitzer (2000) has also used a somewhat similar procedure for computer modeling of molecular structures. A 2D structure is drawn first by hand, linking aliphatic chains covalently with isolated *n*-alkyl aromatic rings. Oxygen-containing compounds included in the structure are carboxyls, phenolic- and alcoholic-hydroxyls, esters, ethers, and ketones. The N content is represented by nitriles and N-containing heterocyclic compounds. The resulting carbon skeleton, presumably of a humic acid monomer, has an elemental composition of 66.8% C, 6.0% H, 26.0% O, and 1.3% N, with a molecular mass of 5540 Da. This is not much different from the monomeric compound previously used by Schulten. This two-dimensional structure is converted into a 3D model with the aid of HyperChem software.

Although all these efforts are indeed brilliantly executed and state-of-the-art modeling of humic acid structures, it is, however, unfortunate that compounds are used, which hardly qualify to be called humic acid. Elemental compositions, calculated from extracted soil humic acids, are available in the literature and have even been presented by Schulten (1994) and Schnitzer (1994). They can be applied as easily as fake figures, and the use of such data is preferable than using data of fictitious materials. The application of fictional figures may create more problems rather than alleviating the issue of the alleged lack of molecular structures of humic acids. It would increase the danger of making the critics even more “die-hard” or “hardcore” nonbelievers on the existence of a humic acid composition and structure.

Another version of computer modeling is presented by Hatcher et al. (1994). A Signature Computer program is applied to construct a 3D chemical structure by introducing the C, H, O, and N weight percentages and selected NMR data obtained from an andosol. By asking the program to use benzene, naphthalene, chrysene, carbonyl, and carboxyl units, the authors confess that they have obtained a confusing equation for computing the amount of each unit required to match the analytical data. However, they claim to be able to redefine their equation by applying an isomer generator provided by the Signature program and managed to draw a 3D structure of the humic acid from an andosol. A 2D reproduction is presented as an illustration in Figure 7.6. As can be noticed, the structure is composed only of benzene

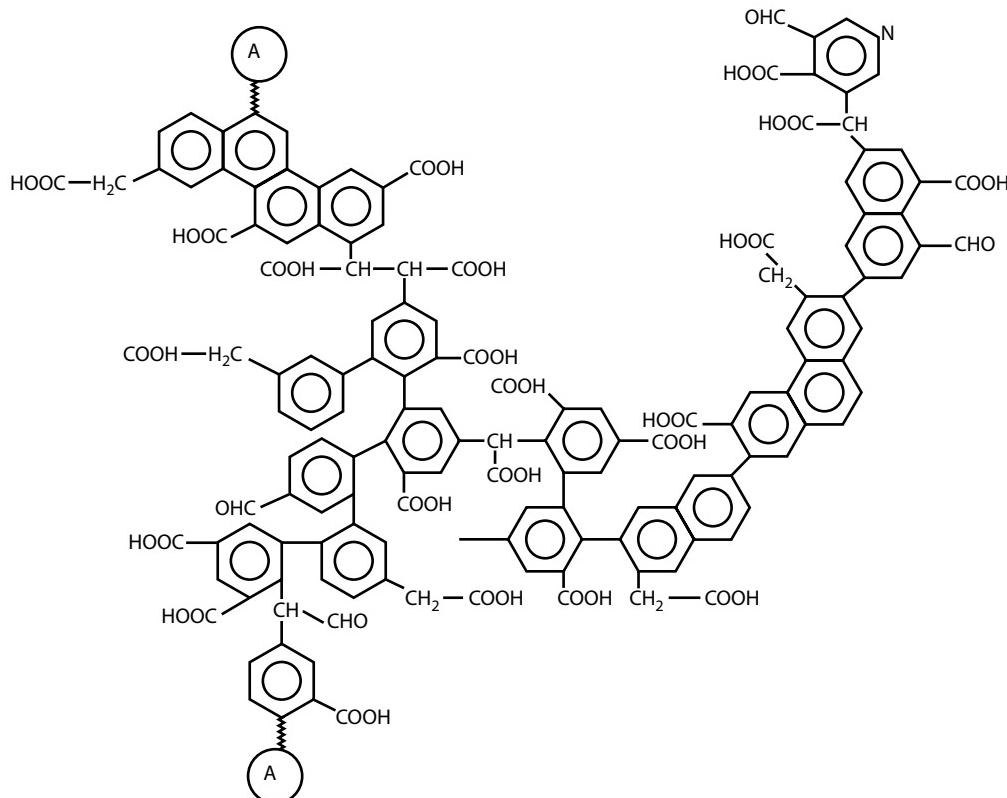


Figure 7.6 Two-dimensional computer model of humic acid from an andosol, showing only aromatic structural components. (Reprinted from *Humic Substances in the Global Environment and Implications on Human Health*, Hatcher, P. G., J.-L. Faulon, D. A. Clifford, and J. P. Matthews, A three-dimensional structural model for humic acids from oxidized soil, Proc. 6th Intern. Meeting of the Intern. Humic Substances Soc., Monopoli, Bari, Italy, September 20–25, 1992, pp. 133–138, Copyright 1994, with permission from Elsevier.)

or phenol units. The functional groups are represented by carboxyl groups and no phenolic-OH groups have been considered. Polysaccharides, and peptides or amino acids, currently also considered important structural constituents of humic acids, have not been taken into account, although %N has been fed into the program. To consider this structure as representing a humic molecule is a matter of conjecture. A basic question also arises whether this is the structure of a humic acid monomer or a humic acid polymer. The authors, apparently, try to justify all these by declaring that their pure aromatic concept may deviate from other theories.

Chapter 8

Characterization of Humic Substances

8.1 Chemical Characterization

Part of the chemical characterization of humic matter has been discussed in the preceding chapter on chemical composition. It was deemed necessary to cover it in separate chapters because of the many issues or topics, making it too long to include them in one chapter. In addition to the characteristics discussed earlier, humic substances also exhibit molecular weights and very distinctive spectroscopic features. Many scientists have tried using ultraviolet-visible, infrared, and nuclear magnetic resonance (NMR) spectroscopy in the identification of various humic substances, with results that are surprisingly reproducible (Orlov, 1985) for materials considered at one time by many to be fake or operational compounds. These remaining characteristics will be discussed in more detail below.

8.2 Molecular Weights

The topic of molecular weights is closely related to the issue of elemental composition and chemical formulas as discussed in Chapter 7. The possession of a formula composition implies the presence of a molecular weight, which is a basic physical property of humic substances of profound importance for their chemical activity. As discussed earlier, elemental composition, chemical formulas, and molecular weights are controversial issues in

humic acid science. The use of molecular weights in characterization of humic substances encounters many problems because of the alleged poly-dispersive nature of these substances. Owing to the latter property, humic substances are believed to exhibit molecular weights varying widely in values, causing many authors to question the existence of humic substances or otherwise consider humic compounds to be very heterogeneous in nature (Felbeck, 1965). With homogeneous macromolecules, many authors claim all particles to have the same molecular weights; however, it is widely known that the molecular weight of humic substances can vary from as low as 1000 for fulvic acids to as much as several thousands or larger for humic acids (Table 8.1). Surprisingly, the data found thus far in the literature show peat humic substances to exhibit the smallest molecular weight values. Especially, peat fulvic acids are reported to have molecular weights in the range of 1000 to 4000 Da, much smaller than that of aquatic fulvic acids. The sample in Table 8.1 identified by "Nordic Ref." is humic matter from Nordic aquatic ecosystems used perhaps as reference of aquatic humic

Table 8.1 General Range of Molecular Weights (in kDa) of Humic Substances Reported in Literature

| | Fulvic Acid | Humic Acid | Humin |
|--------------------------|-------------|------------|-------|
| Soil | 1–10 | 10–100 | 1000 |
| Aquatic | 1–18 | 3–30 | – |
| Nordic Ref. ^a | 6.1 | 19.4 | |
| Peat | 1–4 | 4–22 | |

Source: Bogoslovskiy, V. N., and B. V. Levinskiy. *Agro-Technologies of the Future. Book I. Energens: The New Generation Humates*. Moscow UNITI Publishers, Moscow, Russia, 2006; Shinozuka, T. et al., *J. Mass Spectrom. Soc. Jpn.*, 32(1), 29–32, 2004; Stevenson F. J. *Humus Chemistry. Genesis, Composition, Reactions*, Second Edition, 1994. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Orlov, D. S. *Humus Acids of Soils*. Moscow University Press. Translated from Russian (K. H. Tan, ed.) Amerind Publ., New Delhi, India, 1985; Wershaw, R. L., and D. J. Pinckney, *J. Res. U.S. Geol. Surv.*, 1, 351–366, 1973; Cameron, R. S. et al., *Eur. J. Soil Sci.*, 23 (4), 394–408, 1972; Tan, K. H., and J. E. Giddens, *Geoderma*, 8, 221–299, 1972; Schnitzer, M., and S. U. Khan. *Humic Substances in the Environment*. Marcel Dekker, New York, 1972.

Note: 1 kDa = 1000 Da → molecular weight = 1000 or 1000 g/mol.

^a Perminova, I. V. et al., *Environ. Sci. Technol.*, 37, 2477–2485, 2003.

matter by Perminova et al. (2003). The molecular weights of its fulvic acids and humic acids as cited from the authors above in the order of 6100 and 19,400, respectively, are somewhat comparable to most soil-derived humic substances. However, Piccolo (2001) tends to believe that molecular weights of humic substances are more in the smaller dimensions of 1000 to 2000, values quoted by Piccolo from reports by Scheffer and Ulrich (1960) and Schnitzer and Khan (1972). This is apparently intended to be in line with his ideas of humic matter being complex associations of nonspecific small organic compounds as proposed in his supramolecular theory. Piccolo is of the opinion that large molecular weight values of 30,000 to 50,000 Da or higher for humic acids, as reported by Flaig and Beutelspacher (1951, 1968), are biased toward higher values by the biopolymer concept suggesting formation of humic substances in the form of large polymers. However, Orlov (1985) indicates that humic acids with molecular weights >600,000 are formed by aggregation due to the presence of high concentrations of humic acids, a process he thinks is affected by higher electrolyte contents. Orlov (1985) notes humic acids formed in the most favorable soil conditions, such as in chernozems (mollisols), are more likely higher in molecular weights than humic acids formed in forest soils. Nevertheless, the very wide range, reported in the literature from a few hundreds to >1,000,000, is at issue and discussed in Chapter 3, Section 3.6.1, as a range too large for any of the values to be the characteristic mass of humic substances (see also Piccolo, 2001, 2002; Tan, 2012).

Physically, molecular weights can be expressed in (1) number-average, (2) weight-average, and (3) z-average molecular weights. These types of molecular weights will be explained in more detail below.

8.2.1 Number-Average Molecular Weight, M_n

This is calculated as follows:

$$M_n = \left(\sum nM \right) / n$$

where n = number of component molecules and M = molecular weight of component molecules. The methods used to determine M_n are osmometry, diffusion, and isothermal and cryoscopic distillation. Osmometry is considered the best method but it appears not to be applicable to analysis of molecular weights >200,000.

8.2.2 Weight-Average Molecular Weight, M_w

This is defined as

$$M_w = \left(\sum nM^2 \right) / \left(\sum nM \right)$$

which is usually measured using viscosity analysis and gel filtration. Of the two, gel filtration is the simpler method.

8.2.3 Z-Average Molecular Weight, M_z

This is defined as

$$M_z = \left(\sum nM^3 \right) / \left(\sum nM^2 \right)$$

This is normally measured by the sedimentation method and creates many problems in humic compounds owing to their negative charges balanced by cations creating a diffuse double-layer system. Because of the latter, the molecules tend to repel each other, offsetting the sedimentation

Table 8.2 Differences in Molecular Weight Values (in kDa) Expressed as M_n , M_w , and M_z Reported in Literature

| | M_n | M_w | M_z |
|----------------------|-----------|------------|-------------|
| Soil humic acids | 6.3–2100 | 24.0–230.0 | 126.0–450.0 |
| Soil humic acids | 4.2–8.6 | 14.7–20.4 | |
| Soil fulvic acids | 4.0–6.8 | 9.0–13.9 | |
| Peat humic acids | 17.0–47.0 | >48.0 | >156.0 |
| Peat humic acids | 0.7–0.8 | 4.5–6.8 | |
| Aquatic humic acids | 4.2–7.6 | 8.8–16.1 | |
| Aquatic fulvic acids | 5.3–7.1 | 10.8–13.1 | |

Source: Song, J. H. et al., *Soil Sci. Soc. Am. J.*, 74(6), 2013–2020, 2010; Perminova, I. V. et al., *Environ. Sci. Technol.*, 37, 2477–2485, 2003; Stevenson, F. J.: *Humus Chemistry. Genesis, Composition, Reactions*. Second Edition. 1994. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Note: 1 kDa = 1000 Da → molecular weight = 1000 or 1000 g/mol.

process. Intermolecular repulsion yields high-diffusion and low-sedimentation coefficients owing to faster sedimentation of the larger molecules than the counterions, resulting in an electrostatic drag. In addition, the polydisperse nature makes it difficult to achieve well-defined sediment boundaries with humic substances.

For the study and characterization of humic substances, it is common to use M_w because of its simple determination by filtration. However, for a homogeneous or monodisperse system, $M_n = M_w = M_z$; for a heterogeneous or polydisperse system, $M_n < M_w < M_z$. The data in Table 8.2—compiled from the literature and listed as averages in the table—show humic substances from different sources to increase in molecular weight values from M_n to M_w —in some cases, or generally, also to M_z —suggesting them to be heterogeneous or polydisperse in nature.

8.2.4 Use of Molecular Weight for Characterization

8.2.4.1 Limitations Due to Methods of Analyses

It is perhaps clear by now that humic substances exhibit a wide range of molecular weight values. The differences in values can be attributed to many factors, among which the following two are perhaps the most significant: (1) limitations of determinations and (2) inherent variability of humic substances.

Differences in methods of determination have been widely suspected to have affected the wide variations in molecular weight values. These differences produced by the older different analytical procedures are summarized by Stevenson (1994) to range from 640 to 1,000,000. Vapor pressure-freezing point, osmotic pressure, and x-ray diffraction methods yield the lowest molecular weight values, whereas small angle x-ray scanning and ultracentrifugation methods—in particular sedimentation–diffusion and equilibrium sedimentation—are listed as producing the highest values, in the order of 25,000 to 1,000,000. The concern is raised that the older methods above are unsuitable for use with polydisperse systems, such as humic substances (Piccolo, 2001). Because of their polydisperse nature, Piccolo believes that humic substances may exhibit several diffusion coefficients and sedimentation constants at different particle sizes. The author above claims that the multiple coefficients or constants may account for the wide variations in molecular weights. To justify his claims, Piccolo cites as examples the variations from 77,000 to 2050 Da as determined by Flaig and Beutelspacher (1968) by the ultracentrifuge (sedimentation) method.

Regardless of the above, all efforts in disqualifying the reported values as a specific property characterizing humic substances seem to be based on considering the various values as the results of relatively "comparable" analyses. With this in mind, the following discussion is presented below on the issue. As indicated earlier, values reported for average molecular weights of humic matter may vary from 1000 to 30,000. Flaig and Beutelspacher (1951) state molecular weights of >100,000, and values of 2 million have been reported occasionally. Apparently any number within these ranges can be obtained, depending on the filtration procedures employed, with fulvic acids usually exhibiting the lower and humic acids the higher molecular weight values. Ultrafiltration by Lobartini et al. (1997) with an Amicon cell, employing a membrane with a 10,000 Da exclusion limit at the start, also indicates that humic acid would yield molecular weight fractions as imposed by any exclusion limits used in the analysis. However, the elemental composition, infrared spectra, and electron micrographs show that the different fractions obtained contain essentially the same components, suggesting a composition more homogeneous in nature than previously expected.

The methods of filtration and gel chromatography in fact measure molecular weight ranges, rather than the weight-average molecular weights or the mean values. By means of molecular filtration using gels with a series of exclusion limits, a range of molecular weight values from 2600 to 1,360,000 have been reported (Cameron et al., 1972). However, Stevenson (1994) is of the opinion that the most abundant part of the molecular weight distribution is around 100,000 and assumes that the highest value recorded of 1,360,000 is caused by formation of aggregates or attributed to an extended molecular weight tail. He believes that the upper molecular weight average of humic acids is approximately 200,000 Da and the lower limits are perhaps in the range of 50,000 to 70,000. Stevenson's opinion about aggregation is in fact not new but is only supporting Orlov's (1985) idea, indicating earlier that at high concentrations humic particles tend to aggregate into huge molecules, a process affected by high electrolyte contents. Therefore, the foregoing discussion would tend to indicate that the high molecular weight values are then the product of analyses with humic aggregates. This is supported by the supramolecular theory with its concept of associations of small organic compounds aggregating randomly into large humic entities (Piccolo, 2001). The likelihood that in the case of molecular weights >1,000,000 large humic particles aggregating from small substances are analyzed is corroborated by Piccolo (2001).

8.2.4.2 Controversy of Size Exclusion Chromatography

Today, size exclusion chromatography, which in fact is only a new name for the old gel filtration methods as discussed above, has attracted a lot of attention since it has been used, lately, extensively by a great number of scientists. Published under impressive names such as LPSEC and HPSEC, for low-pressure or low-performance and high-pressure or high-performance size exclusion chromatography, respectively, the method seems to have become the most popular method of the century (Song et al., 2010; Perminova et al., 2003; Piccolo, 2001, 2002), although yielding meager or mixed results. Some have even combined the method with mass spectrometry and given a most imposing name: MALDI–TOF–MS for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Shinozuka et al., 2004). The analysis conducted, with only a limited number of samples, e.g., two soil and two peat samples, reveals number-average molecular weight values of 3700, 2950, 19,500, and 9700 for peat-HA, peat-FA, soil-HA, and soil-FA, respectively. These results only support established knowledge of fulvic acids being lower in molecular weight than humic acids, and peat humic matter being smaller in molecular weight than soil humic substances (see Table 8.1). In addition to the above, it seems necessary to reinforce the fact that size exclusion methods depend on the use of a particular gel with a specific molecular weight size limit (Sephadex and the like) functioning as a filter. As discussed earlier in Chapter 3, Section 3.6.2, these modern highly acclaimed LPSEC and HPSEC methods would, therefore, yield any molecular weight fractions as imposed by any size exclusion limit used in the analysis. Hence, the various sizes of humic fractions obtained are only operational or fake molecular sizes.

8.2.5 Relationship between Molecular Weight, Size, and Shape

8.2.5.1 Molecular Size versus Molecular Weight

From the results of filtration analysis using Sephadex gels—the present-day LPSEC or HPSEC methods—with different size exclusion limits, Tan and McCreery (1975) note that the degree of polymerization and the sizes of the molecules isolated affect the molecular weights of humic matter. A summary of the data listed in Table 8.3 demonstrates the relation between the size of the molecule and molecular weight. By assuming that the humic molecules are spherical in shape, the larger the size of the molecule of the humic

Table 8.3 Molecular Weights and Size (in Å and nm) of Humic Acids Obtained by Sephadex Gel Filtration

| Molecular Weight | Molecular Volume, Å | Radius | |
|------------------|---------------------|--------|------|
| | | Å | nm |
| 30,000 | 23,622 | 17.8 | 1.78 |
| 5000 | 3937 | 9.8 | 0.98 |
| 1500 | 1181 | 6.6 | 0.66 |
| 1000 | 787 | 5.7 | 0.57 |

Source: Tan, K. H., and R. M. McCreery. Humic acid complex formation and intermicellar adsorption by bentonite. Proc. Int. Clay Conf. Mexico City, Mexico, July 16–23, 1975, S. W. Bailey (ed-in-chief). Applied Publ. Ltd., Wilmette, IL, pp. 629–641, 1975; Tan, K. H. *Environmental Soil Science*, Second Edition. Marcel Dekker, New York, 2000.

compound isolated, the larger will be the numerical value of the average molecular weight of humic acid.

8.2.5.2 Issue of Molecular Shape

The shape of humic molecules is also an issue since it may vary considerably from author to author. A number of variations in shapes have been reported, from spheroidal or globular to ellipsoidal, from flexible linear to folding chains and random coil structures, and more (Mukherjee and Lahiri, 1959; Visser, 1964; Gosh and Mukherjee, 1971; Orlov, 1985). With the new micellar and nanotube theories, shapes like micelles, membranes, nanotube fibers, and nanotube membranes, as discussed in Chapter 3, Section 3.7.3, are adding to the complexity, making the issue more confusing than it already is. Evidence has been presented by the present author, through electron microscopy, about the shape of fulvic acid molecules in the form of a fishnet or chicken-wire pattern (Figure 3.3), resembling the basic carbon nanotube structure of graphene. In a more recent investigation, the stunning discovery is made by Tan (2011b) of humic acid molecules, extracted from lignite, to exhibit the characteristic honeycomb pattern, indicative of the presence of nanotube membranes (Figure 5.2). Interesting to note is that in older electron microscopic analyses, Schnitzer and coworkers in Canada and Tan in the United States reveal consistently the long fiber-like and sheet-like

(micelle-like) structures of humic molecules, supporting the micelle and nanotube membrane theories (Chen and Schnitzer, 1976; Tan, 1985).

8.2.5.3 Frictional Ratio and Humic Particle Shape

Particle shape can be determined by calculating the so-called frictional ratio, which is defined as f/f_o , in which f = frictional coefficient and f_o = frictional coefficient of an unsolvated sphere of the same mass (Cameron et al., 1972; Ritchie and Posner, 1982). These coefficients are calculated using the following equations:

$$f = \frac{RTs}{M} \quad (8.1)$$

where R = gas constant, T = absolute temperature, s = sedimentation coefficient, and M = molecular weight.

$$f_o = 6\pi\eta (3M\nu/4\pi N)^{1/3} \quad (8.2)$$

where η = viscosity, ν = partial specific volume of colloid, and N = Avogadro's number.

The values of f/f_o or frictional ratios are unity (equal to 1), as reported by Flraig and Beutelspacher (1968). This is the reason for considering the humic molecules to be spherical or globular in shape. The ratio will exceed unity for shapes differing from spheres or when an interaction takes place between the humic molecule and the solvent. However, more recent observations indicate that the frictional ratios may increase with molecular weight, as can be noticed from the data listed in Table 8.4. High values for f/f_o of 1.4 to 2.4 are exhibited by humic acids with molecular weights between 20,000 and 1,360,000, whereas low values of 1.14 and 1.28 are displayed by humic acids with low molecular weights of 2600 and 4400, respectively. Considering standard errors and variations, these low f/f_o values can be taken as approaching unity; hence, they may perhaps indicate that the humic molecules are spherical in shape. Judging from the data in the table, it can be expected for certain that this is true for humic molecules with molecular weights <2000. At the higher molecular weights, the humic acid molecules are believed to have shapes in the form of random coils (Cameron et al., 1972). They are conceived to be negatively charged branched threads that coil and wind randomly with respect to time and space. Coil

Table 8.4 Relation between Frictional Coefficients and Molecular Weights of Humic Acid

| Molecular Weight | f/f_o |
|------------------|---------|
| 2600 | 1.14 |
| 4400 | 1.28 |
| 12,800 | 1.41 |
| 20,400 | 1.46 |
| 20,400 (pH 11) | 1.39 |
| 23,800 | 1.52 |
| 23,900 (pH 7.0) | 1.36 |
| 83,000 | 1.96 |
| 108,300 (pH 11) | 1.42 |
| 125,900 (pH 9.0) | 1.44 |
| 127,000 | 2.18 |
| 412,000 | 2.12 |
| 1,360,000 | 2.41 |

Source: Cameron, R. S. et al., *Eur. J. Soil Sci.*, 23(4), 394–408, 1972; Ritchie, G. S. P., and A. M. Posner, *J. Soil Sci.*, 33, 233–247, 1982.

density is envisioned to increase with branching, yielding shapes of the more compact spherical types than the linear types. The solvent is trapped within the internal regions but can move freely in the peripheral areas. From their studies with surface pressure and viscosity measurements, Gosh and Schnitzer (1980a) believe that humic and fulvic acids behave like rigid *spherocolloids* at high sample concentration, at low pH, or in the presence of sufficient amounts of neutral electrolytes. At low sample concentrations, they are flexible linear colloids.

8.3 Ultraviolet and Visible Light Spectrophotometry

The color of humic substances in solutions is a physical property that has attracted the attention of many scientists who have attempted to use it for

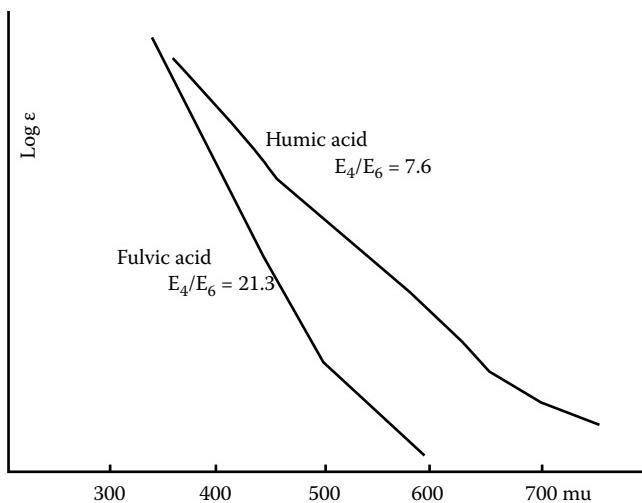


Figure 8.1 Visible light absorption spectra of humic and fulvic acids extracted from an oxisol in the humid tropics.

the characterization of these substances (Flaig et al., 1975; Tan and Van Schuylenborgh, 1961; Schnitzer, 1971; Tan and Giddens, 1972; Kumada, 1987). In Germany, especially, color properties of humic substances have been investigated by a number of scientists, who are of the opinion that the intensity of light absorption was characteristic of the type and molecular weight of humic substances. The absorbance or extinction of humic matter is recorded at various wavelengths from 300 to 800 nm. By plotting the logarithm of the absorbances against the wavelengths, a straight line is usually obtained (Figure 8.1). The slope of such a line is used for differentiation of humic substances, and its importance as a humification index has been discussed in Chapter 5. Fulvic acids are noticed to yield spectra with steep slopes, in contrast to humic acids.

8.3.1 Significance of Color Ratios

As explained earlier, the slope of the spectral curve can be expressed as a ratio or quotient of the absorbances at two arbitrarily selected wavelengths. Many scientists choose the absorbance or extinction values at 400 and 600 nm, and the formula of the ratio, designated as E_4/E_6 or $Q_{4/6}$, called *color ratio*, is given earlier as Equation 5.9. Other scientists opt to use extinction values at 465 and 650 nm, and the ratio is formulated as D_4/D_6 , where D

stands for *optical density*. Orlov (1985) is even of the opinion that the coefficient of extinction, E, can be used for characterization of humic substances.

This color ratio is used as an index for the rate of light absorption in the visible range. A high color ratio, 7 to 8 or higher, corresponds to curves with steep slopes and is usually observed for fulvic acids or humic acids of relatively low molecular weights. On the other hand, a low color ratio, 3 to 5, corresponds to curves that are less steep. These curves are exhibited by humic acids and other related compounds with high molecular weights. The data in Table 8.5 show some E_4/E_6 ratios of humic substances extracted from temperate region soils. It can be noticed that humic acids with high molecular weights (m.w. >30,000) have lower E_4/E_6 values (4.32–4.45) than humic acids with lower molecular weights (m.w. = 15,000). The lower molecular weight humic acids exhibit E_4/E_6 values of 5.47 to 5.49. This is supported by data from

Table 8.5 Color Ratios, E_4/E_6 , of Humic Substances Extracted from Temperate Region Soils

| Soil | Humic Substance | E_4/E_6 |
|--|---------------------------|-----------|
| Ultisols (Cecil soil) ^a | Humic acid, m.w. >30,000 | 4.32 |
| Ultisols (Greenville soil) | Humic acid, m.w. >30,000 | 4.45 |
| Ultisols (Cecil soil) | Humic acid, m.w. = 15,000 | 5.49 |
| Ultisols (Greenville soil) | Humic acid, m.w. = 15,000 | 5.47 |
| Alfisols ^b | Humic acid | 3.5 |
| Andosols ^c | Humic acid | 3.4 |
| Aridisols ^b | Humic acid | 4.3 |
| Mollisols (chernozem) ^b | Humic acid | 3.3 |
| Mollisols (chestnut soil) ^b | Humic acid | 3.9 |
| Spodosols ^b | Humic acid | 5.0 |
| Ultisols (Cecil soil) ^d | Fulvic acid | 8.0 |
| Unknown ^b | Fulvic acids | 6.0–8.0 |

Source: Data from ^aTan, K. H. *Environmental Soil Science*, Second Edition. Marcel Dekker, New York, 2000; ^bSchnitzer, M., and S. U. Khan. *Humic Substances in the Environment*. Marcel Dekker, New York, 1972; Kononova, M. M. *Soil Organic Matter*. Pergamon Press, Elmsford, NY, 1966; ^cKumada, K. *Chemistry of Soil Organic Matter*. Japan Sci. Soc. Press, Tokyo, 1987; ^dTan, K. H., and J. E. Giddens, *Geoderma* 8: 221–229, 1972.

the literature, which, in general, show humic acids to be characterized by E_4/E_6 ratios between 3.3 and 5.0 in contrast to fulvic acids whose E_4/E_6 ratios are between 6.0 and 8.0. The values of D_4/D_6 , as reported by Orlov (1985), seem also to agree by showing a range of 4.1 to 4.8 for humic acids as compared with a range of 9.0 to 17.7 for fulvic acids. The corresponding E values are higher for humic acids (0.061–0.104) than for fulvic acids (0.010–0.016). These observations are not in conformity with Orlov's assumption that the E value is related to the molecular weight of humic acid, which he formulated as follows:

$$E = \frac{\epsilon}{MW \times 100} \quad (8.3)$$

$$MW = \frac{\epsilon}{E \times 100} \quad (8.4)$$

where MW = molecular weight, ϵ = molar coefficient of absorption, and E = measured extinction coefficient.

Equation 8.4 indicates that the value of MW increases when E decreases (at constant ϵ). Similarly the value for MW decreases when E increases. Fulvic acids are noticed to be substantially lower in E values than their humic acid counterparts, hence should exhibit higher molecular weight values if Equations 8.3 and 8.4 are valid assumptions.

8.3.1.1 Controversy of Visible Light Spectrophotometry and Proposed Alternatives

Concerns on the significance of the use of E_4/E_6 ratios have also been expressed by Piccolo (2001) who has listed a series of objections derived from his interpretations of published reports. He claims that it is difficult to reconcile the usefulness of color indices with a report showing that humic matter with the lowest mean residence time exhibits high E_4/E_6 ratios.

Piccolo also believes that an article—suggesting humic substances with large molecular weights and low E_4/E_6 ratios to be aliphatic in composition—is also not affirmative with the concept of color ratios as indices of high or low molecular weights. However, in the end, the author admits that E_4/E_6 ratios can be upheld when highly purified humic acids are used in the analyses.

Since UV-visible light spectra of humic compounds are generally featureless straight lines, Salfeld (1975) suggests modifying the analysis by

measuring the absorbances at intervals of 10 nm in the range of 230 to 700 nm. The difference between two adjacent absorbances ($\equiv \Delta E$) is considered to reflect the slope of the curve. By plotting the logarithms of ΔE against the wavelengths, a curve is obtained with several peaks, called the *derivative spectrum*. Another simpler variation to express the inclination of the spectral lines is the method of Kumada (1987), who uses $\Delta \log k$ values, which have been defined and discussed in Chapter 5.

Perhaps it is also important to mention that humic compounds can also be characterized by fluorescence spectra. By using fluorescence excitation spectroscopy, Gosh and Schnitzer (1980b) show both fulvic and humic acids to yield spectral curves with distinctive bands at 465 nm. Fulvic acid appears to distinguish itself from humic acids by displaying an additional band at 360 nm.

8.3.2 Application of the Bouger–Lambert–Beer Law

Finally, mention should also be made briefly that colorimetric analysis of humic acid solutions obeys the *Bouger–Lambert–Beer law* (Orlov, 1985; Tan, 1996), hence provides applicabilities for measurements of the concentrations of humic substances. This law is usually formulated as follows:

$$\log (I_o/I_t) = D = \epsilon lc \quad (8.5)$$

where I_o = intensity of incident light, I_t = intensity of transmitted light, ϵ = extinction coefficient, l = thickness of sample, and c = concentration.

By using a sample holder of 1 cm thickness, $l = 1.0$, the law above indicates that the optical density or absorbance is directly proportional to ϵc ; in other words, to concentrations:

$$\log (I_o/I_t) = D = \epsilon c \quad (8.6)$$

Conformity to Lambert's and Beer's law gives a linear regression if optical density or absorbance is plotted against concentrations (Tan, 1996). However, the few colorimetric procedures presented in the literature for a rapid quantitative determination of humic acid have been accepted only with mixed blessings. The method proposed by Holmgren and Holzhey (1984), using 2-amino-2-methyl propanol buffer, is apparently based on measurement of color related to the amount of Fe and Al chelated by the humic substances.

8.4 Infrared Spectroscopy

This method is another important and relatively simple-to-use tool in the characterization of humic substances. Infrared spectroscopy has been used extensively in the past to characterize humic substances, although some doubt exists about the significance of the infrared spectra. Spectroscopic methods in general are deemed by MacCarthy and Rice (1985) to be severely limited in the study of humic substances. However, of the several spectroscopic methods available, e.g., UV-visible, spectrofluorometry, and electron spin resonance (ESR) spectroscopy, it is the opinion of the authors above that infrared spectroscopy is by far the most useful. Reservations for infrared analysis are perhaps caused in part by the complexity of the infrared spectra of humic preparations. Humic substances are mixtures of polyelectrolytic molecules, and their spectra reflect the responses of the many different molecular species. The use of poorly prepared humic samples and the publication of poorly resolved spectra have aggravated the problem immensely. Allowing the dissemination of infrared spectra with poor resolutions does not do any favors to humic acid chemistry. In spite of these issues, infrared analysis has proven to be very useful. It is deemed to be very valuable in the identification of (1) functional groups and their structural arrangements in the humic molecule, and (2) organic and inorganic impurities. Several typical vibrations of C–H and oxygen-containing functional groups absorb light in the infrared region, yielding peaks, called *absorption bands*, characterizing the spectrum. For those interested in the basic principles of infrared vibration properties and analytical procedures, reference is made to Tan (1996, 2000), Stevenson (1994), MacCarthy and Rice (1985), and Schnitzer (1965).

8.4.1 Infrared Spectra of Humic Matter

Although several procedures are available in infrared analysis of humic substances, the most commonly applied method is the pressed KBr pellet technique (Tan, 1996). The humic matter is mixed with KBr and pressed into a transparent pellet, which is scanned from 4000 to 600 cm⁻¹. Sometimes scanning is continued to 400 cm⁻¹, but frequently the characteristic infrared bands are located mostly within 4000–600 cm⁻¹ (Table 8.6). The spectrum is often divided into two regions:

1. A group-frequency region (4000–1300 cm⁻¹)
2. A fingerprint region (1300–650 cm⁻¹)

Table 8.6 Infrared Absorption Bands of Functional Groups in Humic Matter

| Wave Number, cm^{-1} | Wavelength, μm | Proposed Assignment |
|-------------------------------|---------------------------|--|
| 3400–3300 | 3.94–3.03 | O–H and N–H stretch |
| 3380 | 2.950 | Hydrogen bonded OH |
| 2985 | 3.35 | CH_3 and CH_2 stretch |
| 2940–2900 | 3.40–3.44 | Aliphatic C–H stretch |
| 1725–1720 | 5.79–5.81 | C=O stretch of COOH groups |
| 1650–1630 | 6.00–6.10 | C=O stretch (amide I), aromatic C=C, hydrogen bonded C=O, double bond conjugated with carbonyl and COO^- vibrations |
| 1650–1613 | 6.00–6.19 | COO^- symmetrical stretch |
| 1460 | 6.85 | Aliphatic C–H, CC– H_3 |
| 1440 | 6.95 | C–H stretch of methyl groups |
| 1435 | 6.97 | C–H bending |
| 1400 | 7.14 | COO^- antisymmetrical stretch |
| 1390 | 7.20 | Salts of COOH |
| 1280–1230 | 7.80–8.10 | C–O stretch, aromatic C–O, C–O ester linkage, phenolic C–OH |
| 1170–950 | 8.50–10.5 | C–C, C–OH, C–O–C typical of glucosidic linkages, Si–O impurities, C–O stretch of polysaccharides |
| 1035 | 9.67 | O– CH_3 vibrations |
| 840 | 11.9 | Aromatic C–H vibrations |

Source: Tan, K. H., *Soil Sampling, Preparation, and Analysis*. Marcel Dekker Inc., New York, 1996; Tan, K. H., *Environmental Soil Science*, Second Edition. Marcel Dekker, New York, 2000. Stevenson, F. J.: *Humus Chemistry, Genesis, Composition, Reactions*. 1982. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Stevenson, F. J.: *Humus Chemistry, Genesis, Composition, Reactions*, Second Edition. 1994. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Mortenson, J. L., D. M. Anderson, and J. L. White. Infrared spectroscopy. In: *Methods of Soil Analysis. Part I*, C. A. Black (ed-in-chief). Agronomy Series No. 9. Am. Soc. Agronomy Inc., Publ., Madison, WI, pp. 743–770, 1965.

In the group-frequency region, the principal bands may be assigned to vibration units that consist of only two atoms to a molecule. In the fingerprint region, single bond stretching and bending vibrations of polyatomic systems are major features. Molecules similar in structure may absorb similarly in the group-frequency region but will show differences in absorption in the fingerprint region.

Notwithstanding the many arguments on the usefulness of infrared analysis of humic substances, the method is capable of detecting and distinguishing between the different types of humic substances and organic compounds in general. Examples of spectra are given in Figure 8.2 as illustrations. Normally, the shape of the whole spectrum from 4000 to 600 cm^{-1} is used as a “fingerprint” for the determination of a particular type of humic substance.

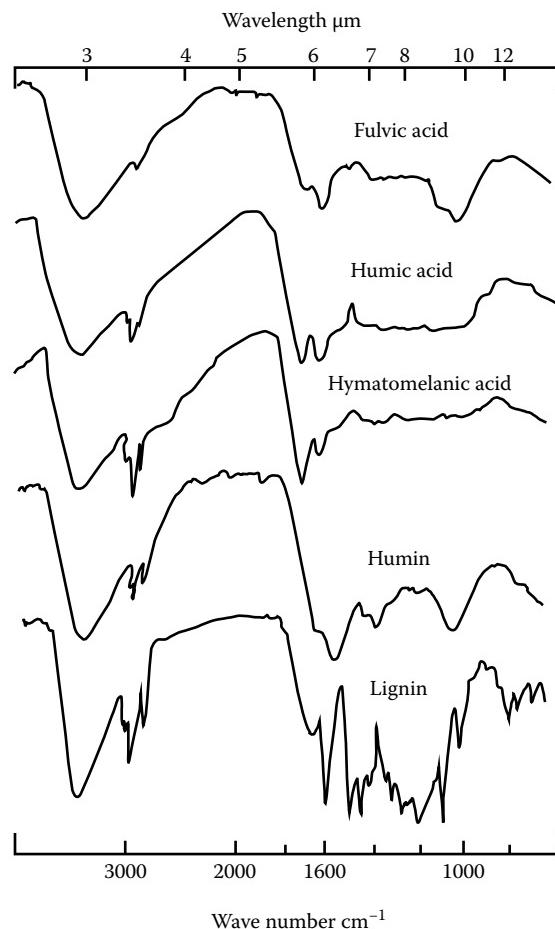


Figure 8.2 Characteristic spectra of major humic substances and lignin.

8.4.1.1 Fulvic Acid Spectrum

As can be noticed in Figure 8.2, the spectrum of fulvic acid has very different infrared absorption features than humic acids or the other substances, hence as indicated above can be used as a fingerprint for identification purposes. The fulvic acid spectrum has a strong absorption band at 3400 cm^{-1} , a weak band between 2980 and 2920 cm^{-1} , a shoulder at 1720 cm^{-1} followed by a strong band at 1650 cm^{-1} , and a strong band at 1000 cm^{-1} . These bands are attributed to vibrations of OH; aliphatic C–H; carbonyl (C=O) followed by carboxyls in COO^- form; and ethyl, vinyl ($-\text{CH}-\text{CH}_2$), aromatic aldehyde, amine, and SH groups, respectively. This infrared spectrum shows close similarities to the infrared spectrum of polysaccharides (Tan and Clark, 1968).

Additional fulvic acid spectra collected from a great number of scientists, for the purpose of comparison with the spectrum used as a “standard” in Figure 8.2, are compiled in Figure 8.3. All the spectra, as a whole, agree closely with the shape of the spectrum of fulvic acid used as a “standard.” Perhaps the first spectrum, although showing broadly the infrared absorption features of fulvic acid as in the standard, is an example of a poorly resolved spectrum. Reviewers accepting it for publication, because of bias toward the impressive clout carried by world-renowned scientists, only add to the confusion and the distrust in infrared analyses. Contrary to the belief of most critics, infrared absorption analyses are otherwise the simplest and easiest methods for characterizing fulvic and humic acids.

8.4.1.2 Humic Acid Spectrum

In contrast to fulvic acid, the humic acid spectrum is characterized by a strong aliphatic C–H absorption band between 2980 and 2920 cm^{-1} and two strong absorption bands for carbonyls and carboxyls in COO^- at 1720 and 1650 cm^{-1} , respectively. In addition, the humic acid spectrum lacks the strong band at 1000 cm^{-1} . This feature frequently distinguishes it from fulvic acid. The presence of a band at 1000 cm^{-1} in a humic acid spectrum is ordinarily associated with impurities with SiO_2 . Such an impurity can be removed by washing the humic acid specimen with a dilute HCl–HF mixture. Some humic acids, especially those extracted from ultisols, may not exhibit the two bands at 1720 and 1650 cm^{-1} , respectively, but may have spectra featuring only the strong band at 1650 cm^{-1} .

Most of the additional spectra listed in Figure 8.4 agree fairly well in infrared absorption features with those exhibited by the humic acid sample

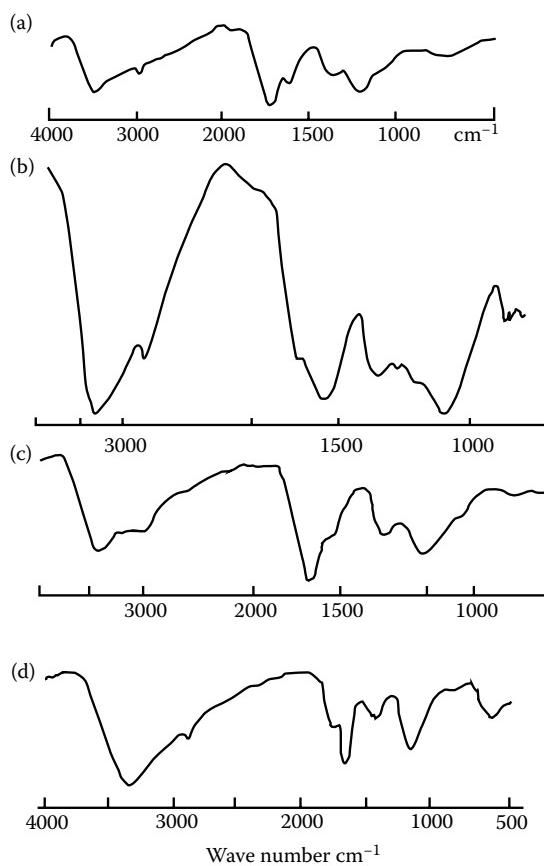


Figure 8.3 Infrared spectra of fulvic acids from different sources as recorded by (a) Schnitzer, (b) MacCarthy and Rice, (c) Stevenson, type II, (d) Kemp and Mudrochova. (From Schnitzer, M. Chemical, spectroscopic, and thermal methods for the classification and characterization of humic substances. In: *Humic Substances. Their Structure and Function in the Biosphere*, D. Povoledo, and H. L. Golterman (eds.). Proc. Intern. Meeting Humic Substances, Nieuwersluis, the Netherlands, May 29–31, 1972. Centre for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 293–310, 1975; MacCarthy, P., and J. A. Rice: Spectroscopic methods (other than NMR) for determining functionality in humic substances. In: *Humic Substances in Soil, Sediment, and Water. Geochemistry, Isolation, and Characterization*. 1985. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Stevenson, F. J.: *Humus Chemistry. Genesis, Composition, Reactions*, Second Edition. 1994. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Kemp, A. L. W., and A. Mudrochova. Nitrogen in sedimented organic matter from Lake Ontario. In: *Humic Substances. Their Structure and Function in the Biosphere*, Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Centre for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 137–157, 1975.)

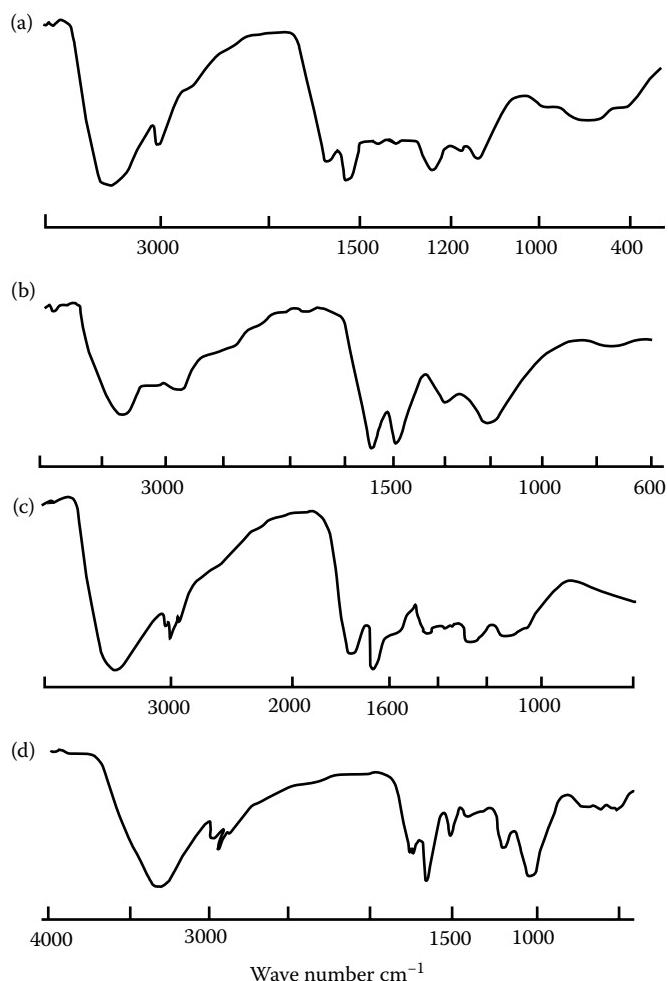


Figure 8.4 Infrared spectra of humic acids from different sources as recorded by (a) Bedrock et al., (b) Stevenson, type I, (c) Tan, and (d) Kemp and Mudrochova. (Reprinted from *Humic Substances in the Global Environment and Implications on Human Health*, N. Senesi, and T. M. Miano (eds.), Bedrock, C. N., M. V. Cheshire, J. A. Chudek, B. A. Goodman, and C. A. Shand, ^{31}P NMR studies of humic acid from a blanket peat, Proc. 6th Intern. Meeting of the Intern. Humic Substances Soc., Monopoli, Bari, Italy, September 20–25, 1992, pp. 227–232, Copyright 1994, with permission from Elsevier; Stevenson, F. J.: *Humus Chemistry. Genesis, Composition, Reactions*, Second Edition. 1994. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission; Tan, K. H., *Plant Soil*, 44, 691–695, 1976; Kemp, A. L. W., and A. Mudrochova. Nitrogen in sedimented organic matter from Lake Ontario. In: *Humic Substances. Their Structure and Function in the Biosphere*, Proc. Intern. Meeting, Nieuwersluis, the Netherlands, May 29–31, 1972. Centre for Agric. Publishing and Documentation, Wageningen, the Netherlands, pp. 137–157, 1975.)

used as a comparative standard in Figure 8.2. The exception is spectrum D, which shows strong absorption bands at 1000 cm^{-1} , which are usually absent in humic acid spectra. Such strong bands are normally exhibited by fulvic acid spectra and are expected to be caused by poor sample preparation, in this case by poor sample purification.

8.4.1.3 Hymatomelanic Acid Spectrum

The infrared spectrum of hymatomelanic acid has very strong absorption bands between 2980 and 2920 cm^{-1} , and at 1750 cm^{-1} , attributed to aliphatic C–H and C=O stretching vibrations, respectively. It has been discovered by Clark and Tan (1969) that hymatomelanic acid is an ester compound formed from humic acid and polysaccharides. This is supported by subsequent investigations by Tan and McCreery (1970) and Tan (1975) that also provide evidence indicating that the C–H group, belonging to the polysaccharides, is esterified to the carboxyl group of the humic acid molecule.

8.4.1.4 Humin Spectrum

The infrared spectrum of humin closely resembles that of fulvic acid. However, a stronger aliphatic C–H absorption between 2980 and 2920 cm^{-1} distinguishes it from the spectrum of fulvic acid. Such close similarities in infrared features of humin and fulvic acid are rather surprising owing to the concept that humin is a condensed form of humic acids.

8.4.1.5 Lignin Spectrum

Lignin has an infrared spectrum that distinguishes it clearly from humic and fulvic acid. Humic matter is believed to be a decomposition product of lignoid or lignin-like compounds.

8.4.2 Classification of Infrared Spectra

Some authors try to group humic matter spectra into several different types. Stevenson has cited Kumada (1987) to have classified infrared spectra into types A, B, R_p , and P. However, these four symbols were used by Kumada (1987) in his book *Chemistry of Soil Organic Matter* for distinguishing types of humic acids by $\Delta \log k$ values obtained from colorimetric analyses. This has been explained earlier several times.

8.4.2.1 Concept of Type I, II, and III Spectra

The infrared data in the preceding section are supplied by Kumada as additional characteristics for the four types of humic acids and not for the purpose of classification of infrared spectra. More real perhaps is the idea of Stevenson and Goh (1971), who have attempted to distinguish the infrared spectra of humic substances into types I, II, and III. *Type I* spectra are the spectra of humic acids, with the absorption bands at 1720 and 1650 cm⁻¹, considered as being equal in intensity. *Type II* spectra are typical for fulvic acids with strong absorption at 1720 cm⁻¹ and weak absorption at 1640 cm⁻¹. The strong band at 1720 cm⁻¹ is attributed by Stevenson (1994) to the occurrence of more COOH groups in fulvic acids than in humic acids. The author also believes that this band at 1720 cm⁻¹ will progressively disappear with an increase in color intensity. These features deviate from those presented for fulvic acids in Figure 8.2, where the band at 1720 cm⁻¹ is very weak and the band at 1640 cm⁻¹ is the strongest and the most prominent band. Most spectra of fulvic acids are of this nature. A strong intensity band at 1640 cm⁻¹ conforms more to the presence of large amounts of carboxyl groups, since this is the absorption band caused by vibrations of carboxyls in COO⁻ form. *Type III* spectra have infrared features similar to type I, but show, in addition, strong bands between 2900 and 2840 cm⁻¹, indicative of more aliphatic C-H compounds.

The infrared spectra reported above by Stevenson and Goh are not much different from those presented in Figure 8.2 and by other authors. Selected spectra, provided for comparison in Figures 8.3 and 8.4, indicate that the three fulvic acid spectra shown do not differ dramatically from each other. The fulvic acid spectrum from MacCarthy and Rice (1994) exhibits a weak shoulder and a strong band at 1720 and 1650 cm⁻¹, respectively, similarly to those reported for the fulvic acid in Figure 8.2. This is supported by the fulvic acid spectrum of Kemp and Mudrochova (1975). The humic acid spectra are also comparable and show little variations from one to the other. The “hump” or broad band near 2900 cm⁻¹ in Stevenson’s type I spectrum is apparently caused by an error in recording, or improper analysis. Usually, humic acid spectra exhibit in this region a series of sharp bands as evidenced by the spectra from Bedrock et al., Kemp and Mudrochova, and Tan. The humic acid spectrum from Kemp and Mudrochova has a strong band at 1000 cm⁻¹. This is usually attributed to impurities by chelated silica, which should have been removed by washing the sample in a dilute HCl–HF

solution before infrared analysis. Humic acid spectra are commonly reported not to have a band at 1000 cm⁻¹.

The humic substances above have been extracted from different soils in different regions and have been analyzed with different models of infrared spectrophotometers. The only thing in common is that they were extracted with the same NaOH procedures. Nevertheless, the similarities of the spectra are apparent. Such reproducibility in infrared spectra, regardless of the different sources, tends to confute the concept of humic substances being fake compounds or artifacts.

8.5 Nuclear Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy in general makes use of magnetic radiation and can be distinguished into two categories: (1) ESR or electron paramagnetic resonance (EPR) and (2) NMR spectroscopy. Electron spin resonance analyzes large free radicals in large polymers of soil organic compounds, and NMR analysis was used in the beginning for the determination of proton resonance in relatively small organic compounds. With the rapid development of the technique, today NMR has been expanded for use with ³¹P, ¹⁹F, ¹³C, ¹⁵N, and ¹¹³Cd (Pfeffer and Gerasimowicz, 1989). For the study of organic carbon in humic substances, ¹³C-NMR spectroscopy is the preferred method. Nitrogen-15- and ³¹P-NMR are suitable for the analysis of organic N and organic P, respectively (Thorn et al., 1996; Lobartini et al., 1989, 1998).

8.5.1 Electron Paramagnetic Resonance

In the study of humic acids, EPR or ESR spectroscopy has been used occasionally, and for several reasons it has been abandoned since the 1980s. The method is rather complicated and requires a lot of calculations of spectroscopic splitting factors, called g-values. In principle, a solid ground sample placed in a quartz tube is analyzed by an ESR spectrometer. The magnetic field at the sample is calibrated relative to diphenylpicrylhydrazyl (DPPH) or another suitable standard, and the spin concentration of the sample is determined by comparison with known concentrations of DPPH diluted with powdered KCl. The differences in field between maxima in the derivative signals are considered as the line widths (G). The

spectroscopic splitting factors (*g*-values) are calculated using the following equation derived from values of the magnetic field (*H*) at which resonance occurs for the sample:

$$\frac{H_2}{H_1} = \frac{g_s}{g_r} \quad (8.7)$$

where H_1 = resonance of sample, H_2 = resonance of reference (standard), g_s = *g*-value of sample, and g_r = *g*-value of reference (DPPH has a $g_r = 2.0036$).

The ESR spectra of humic substances consist of single lines with hyperfine splitting with *g*-values ranging from 2.0031 to 2.0045 and line widths from 2.0 to 3.6 G (Gosh and Schnitzer, 1980b; Riffaldi and Schnitzer, 1972). Examples of ESR spectra from a spodosol and soil humic substances are shown in Figure 8.5. The peak in the spodosol spectrum is identified as the organic radical in the humic acid molecule (Steelink and Tolling, 1967). The spectrum of humic acid supports this observation.

Steelink (1964) and Steelink and Tolling (1967) were perhaps the first to try to apply ESR methods to show paramagnetism in humic acids owing to the presence of semiquinones and hydroxyquinones. This was followed later by Riffaldi and Schnitzer (1972), Senesi and Schnitzer (1977), and Gosh and Schnitzer (1980b), who confirmed by ESR analyses the presence of semiquinone radicals in humic acids.

More recently, ESR spectroscopy has found application in the study of metal chelation by humic substances for the determination of speciation of metals, forming inner sphere complexes difficult to analyze by any other methods. An example of an ESR spectrum of an Fe–fulvic acid complex is shown in Figure 8.5. Three main signals or resonances have been reported in the ESR spectra of naturally occurring Fe–fulvic acid complexes (Senesi et al., 1977):

1. An antisymmetrical resonance at $g = 4.1$ with an average line width of 125 G
2. An isotropic resonance at $g = 2.0028$ to 2.0043 and a line width of 4.3 to 6.4 G
3. A resonance at $g = 2$, composed of poorly defined, broad lines

From these studies, Senesi and coworkers believe that Fe is attracted to fulvic acid as Fe^{3+} by two possible mechanisms: (1) by tetrahedral and/or octahedral coordination bonds and (2) by adsorption on external fulvic acid surfaces.

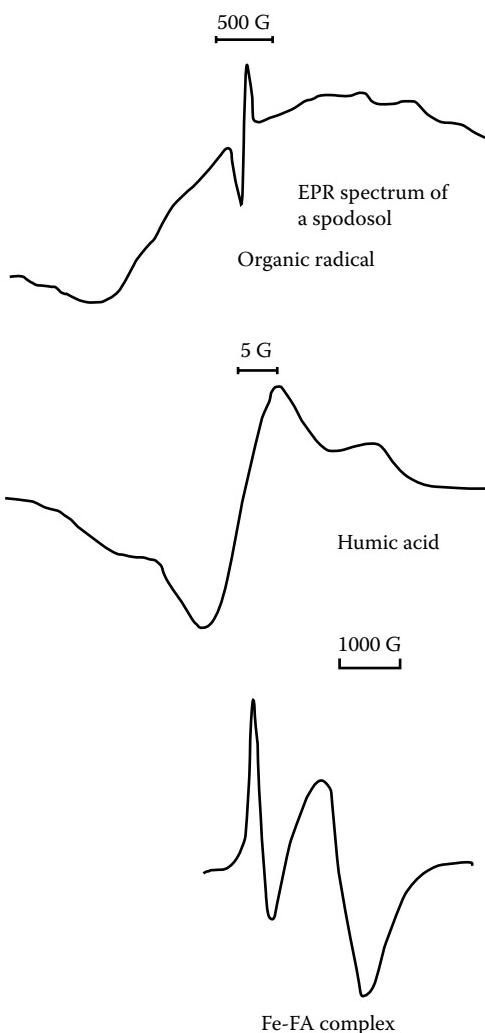


Figure 8.5 Electron spin resonance spectra of a spodosol (top), humic acid (middle), and Fe-fulvic acid complex (bottom). (From Steelink, C., and G. Tolling. Free radicals in soils. In: *Soil Biochemistry*. Marcel Dekker, New York, pp. 147–173, 1967; Schnitzer, M. Binding of humic substances by soil mineral colloids. In: *Interactions of Soil Minerals with Natural Organics and Microbes*. SSSA Special Publ. No. 17. Soil Sci. Soc. Am. Inc. Madison, WI, pp. 77–101, 1986.)

8.5.2 Carbon-13 Nuclear Magnetic Resonance

Of more importance, apparently, is ^{13}C -NMR spectroscopy, which has attracted substantially more attention than ESR, and now is even heralded as the most important method in the study of humic acids. For the basics of

NMR spectroscopy in general and its application in agriculture and humic acids, reference is made to Wilson (1981), Wershaw (1985), Pfeffer and Gerasimowicz (1989), and Bortiatynski et al. (1996). In this section, only the subjects necessary for a proper understanding of an NMR spectrum will be briefly discussed, such as the chemical shift, δ .

Spectroscopic analysis involves interaction of radiation with matter, and that part that is absorbed or emitted by the sample is detected as a function of wavelength or frequency, which is defined as the spectrum. The frequencies in NMR analysis are usually in the range of 100 to 600 MHz, hence associated with relatively closely spaced transitions between energy levels. These levels depend on the different magnetic states of the nucleus and are proportional to the applied magnetic field as can be noticed from the following equation (Pfeffer and Gerasimowicz, 1989):

$$\Delta E = h\gamma B \quad (8.8)$$

where ΔE = difference in energy states, h = Planck's constant, γ = magneto-gyric ratio characteristic of each nucleus, and B = applied magnetic field.

In addition to the applied magnetic field, a small localized magnetic field is present around the nucleus, produced by the electronic currents of the atoms. Because of this, each nucleus within a molecule absorbs energy at a slightly different frequency or resonance position. The separation of these resonance frequencies from a standard (chosen) reference is called the chemical shift, δ , which is usually calculated using the following equation:

$$\delta = \frac{\nu_s - \nu_r}{\nu_r} 10^6 \quad (8.9)$$

where δ = chemical shift in ppm, ν_s = resonance frequency of sample peak, and ν_r = resonance frequency of standard reference peak.

In the NMR spectrum, each signal (peak) is identified by a value of δ . The standard reference in ^{13}C -NMR and ^1H -NMR is tetramethylsilane (TMS), which has a chemical shift, $\delta = 0.0$ ppm.

In its early development, NMR was capable of analyzing only the hydrogen atoms, or protons, of the humic molecules. The usefulness of such analysis is questioned by several scientists because of the lack of success in obtaining spectra of humic substances by ^1H -NMR analysis. In the early days, the main problem was that ^{13}C -NMR analysis required the sample to be dissolved in a suitable solvent. The solvents frequently used at that time were CCl_4 (chloroform) and CDCl_3 . Humic acid, however, is usually not soluble in these

reagents, and must first be methylated or broken down into smaller molecules by degradation procedures. An aqueous medium (water), needed for ^1H -NMR, is unsuitable for ^{13}C -NMR. Solid samples at that time cannot be used since they interfere with magnetic interaction. Another solvent is D_2O , which finds application with analysis of fulvic acids. Today, the use of NaOD has apparently eliminated this obstacle. In addition to the foregoing problems, difficulties also arise from the use of radio waves in NMR analysis, which are low-energy forms of electromagnetic radiation. The level of energy is considered very small, but still large enough to affect the nuclear spin of the atoms in the poorly defined complex polymers of humic acid molecules. This makes an analysis by NMR very time consuming, and even with today's powerful modern machines one analysis may take several hours of scanning time.

Both liquid and solid samples can now be used. Soil samples can also be used in the undisturbed state, provided the sample contains sufficient amounts of organic carbon (Tan, 2000; Tan et al., 1992). With the very powerful instruments available today, cross-polarization magic angle spinning carbon-13 NMR (CP-MAS ^{13}C -NMR) spectroscopy produces better spectra with solid than with liquid samples of humic acids. Magic angle spinning is a technique by which a sample is rapidly rotated at an angle of 54.7° , known as the *magic angle*, in order to decrease line broadening of the spectrum.

The analysis with ^{13}C -NMR spectroscopy is capable of measuring the distribution of C in the various types of compounds, and this information can be used in structural analysis and in differentiating the different types of humic matter. A ^{13}C -NMR spectrum of humic matter can usually be divided into several regions (Hatcher et al., 1980), and the various peaks in the spectrum assigned to specific carbon functional groups. Peak heights and peak areas are used for quantitative measurements by the integration method. This has been discussed earlier in Chapter 6, and to support the capability of ^{13}C -NMR analysis as contended above, the following spectra are provided as illustrations in Figure 8.6. As can be noticed, the spectrum of lignite-humic acid has a strong signal between 105 and 165 ppm chemical shift for aromatic carbon, and a weak signal between 165 and 185 ppm for carboxyl carbon. In contrast, the spectrum of fulvic acid shows a weak signal for aromatic carbon but a substantially strong signal for carboxyl carbon. This is supported by the other two spectra from Suwannee River aquatic humic substances, where the spectrum of humic acid is also dominated by strong signals between 105 and 165 ppm. On the other hand, the aquatic fulvic acid spectrum shows again a weak signal in the aromatic region, but has a very strong peak in the carboxyl region at 177 ppm chemical shift.

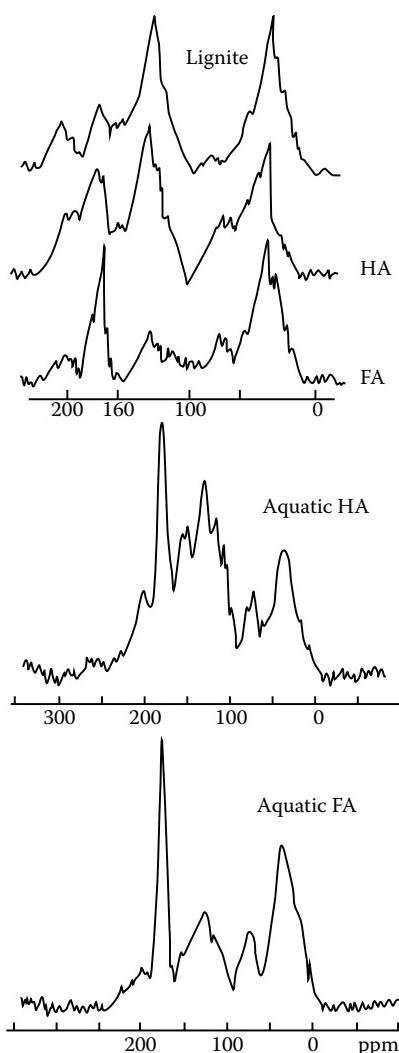


Figure 8.6 Solid-state CP-MAS ^{13}C -NMR spectra of humic and fulvic acids extracted from lignite, and liquid-state ^{13}C -NMR spectra of aquatic humic substances from the Suwannee River. (From Tan, K. H., D. S. Himmelsbach, and J. C. Lobartini, *Comm. Soil Sci. Plant Anal.*, 23, 1513–1532, 1992; Thorn, K. A. Nuclear-magnetic resonance spectrometry investigations of fulvic and humic acids from the Suwannee River. In: *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*. U.S. Geol. Survey Open-File Report 87-557, 1989.)

These observations agree with the chemical data indicating that fulvic acids contain larger amounts of carboxyl groups than humic acids. Additional evidence for these differences between fulvic and humic acids are provided by other spectra shown in Figure 8.7, where the peaks in the aromatic region of

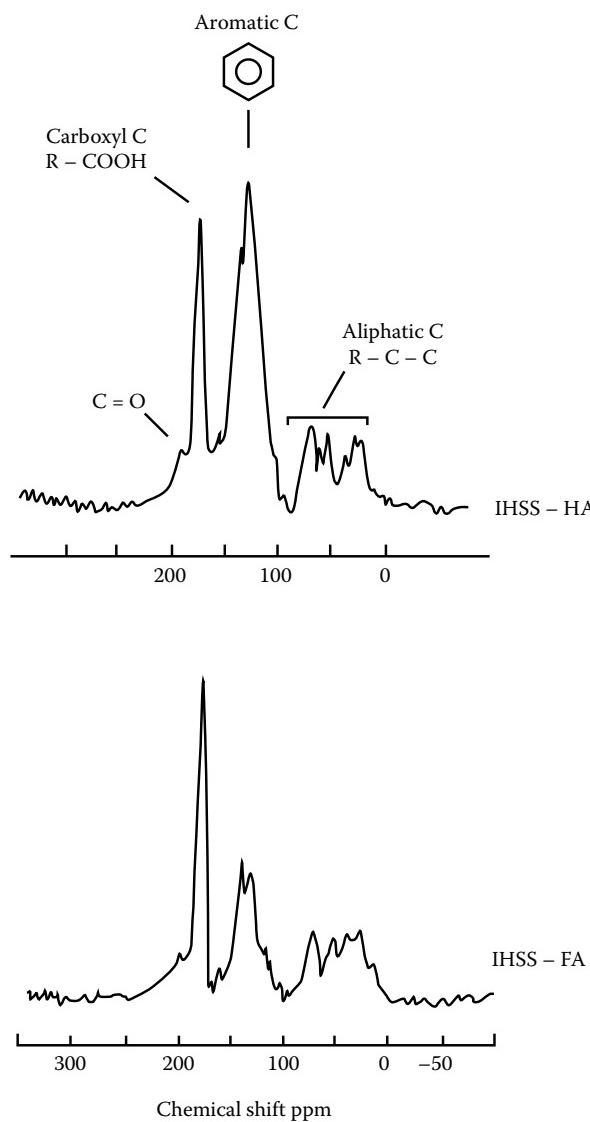


Figure 8.7 Differences in aromaticity between standard humic and fulvic acids from the International Humic Substances Society (IHSS) as revealed by liquid-state ^{13}C -NMR spectroscopy. (Adapted from Thorn, K. A. Nuclear-magnetic resonance spectrometry investigations of fulvic and humic acids from the Suwannee River. In: *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*, U.S. Geol. Survey Open-File Report 87-557, 1989; Thorn, K. A., D. W. Folan, and P. MacCarthy. Characterization of the International Humic Substances Society standard and reference fulvic and humic acids by solution state carbon13 (^{13}C) and hydrogen-1 (^1H) nuclear magnetic resonance spectrometry. Water-Resources Investigations Report 89-4196. U.S. Geol. Survey, Denver, 1989.)

the spectrum of soil fulvic acid are small in intensity relative to those in the spectrum of soil humic acid. Both samples are supposed to be standard or reference samples from the International Humic Substances Society (IHSS).

The data in Figures 8.6 and 8.7 also tend to suggest that these spectral features are reproducible, as was the case with infrared spectra as discussed earlier. The aquatic fulvic and humic spectra reported by Thorn et al. (1989) for the Suwannee River samples (Figure 8.8) are in perfect agreement with those produced by Mills et al. (1996) from samples collected 5 years later

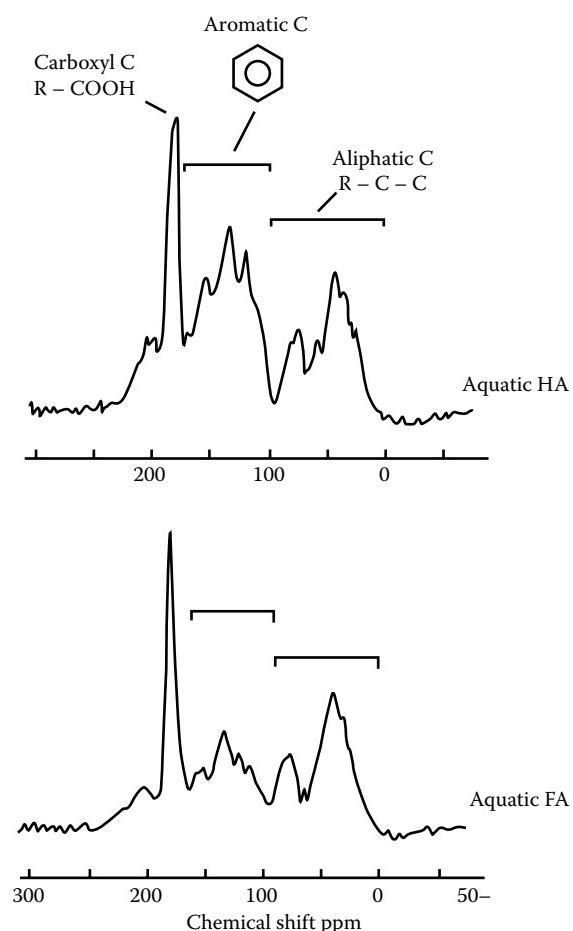


Figure 8.8 Differences in aromaticity between aquatic humic and fulvic acids from the Suwannee River as determined by liquid ^{13}C -NMR spectroscopy. (Reprinted with permission from Mills, M. S., E. M. Thurman, J. Ertel, and K. A. Thorn. Organic geochemistry and sources of natural aquatic foams. In: *Humic and Fulvic Acids. Isolation, Structure, and Environmental Role*, ACS Symposium Series 651, pp. 151–192. Copyright 1996 American Chemical Society.)

from the same river in January 1994 (Figure 8.6). Apparently different handling and techniques also have little influence on reproducibility of spectra as long as the humic substances have been obtained by a similar extraction procedure. The standard IHSS fulvic and humic acid spectra (Figure 8.7) from Thorn et al. (1989) were produced by dissolving in D₂O plus NaOD before ¹³C-NMR analysis, whereas those from Thorn et al. (1996) were dissolved in DMSO, dimethylsulfoxide, an organic extracting reagent for humic substances. Nevertheless, the soil fulvic and humic acid spectra shown in Figure 8.7 are in agreement with those in Figure 8.6, except for the strong DMSO peaks in the latter.

8.5.3 Nitrogen-15 Nuclear Magnetic Resonance

As can be noticed from the discussion above, ¹³C-NMR analysis can only determine qualitatively and quantitatively several major types of carbons, e.g., aliphatic C, polysaccharide C, aromatic C, carboxyl C, and carbonyl C. This method is not capable, however, of measuring the nitrogen content in the nitrogenous constituents of the humic molecule. As discussed in Chapter 5, these constituents are believed to be composed of amino acids, peptides or protein, amino sugars, and HUN (hydrolyzable unknown N), surviving microbial and chemical degradation owing to incorporation in the humic structure by the humification process. The peptides may be linked to the central core of the humic molecule by H-bonding. The conventional way to analyze N compounds in soil and humic acid is by using standard hydrolysis and chromatographic techniques. However, the method is believed to be suspect due to the inherent analytical problems, such as incomplete extraction and losses during hydrolysis (Stevenson, 1994). Using the old methods, only 30% to 50% of the total N in soils can be recovered, mostly as soluble amino acids and amino sugars.

With the recent advancement in NMR spectroscopy, many scientists are now turning their attention to this technique for the analysis of N substances in humic acids. It is a nondestructive method and still in its early stages of development. In this respect, two isotopes are available for application in NMR spectroscopy: ¹⁴N and ¹⁵N. Nitrogen-14 is the most abundant isotope in organic substances but ¹⁴N-NMR analysis has been reported to yield broad line spectra or spectra with poor resolutions. On the other hand, ¹⁵N, with only 0.37% abundance, is less available in nature; however, ¹⁵N-NMR analysis produces spectra with higher resolutions, although it is still considered 50 times less sensitive than ¹³C-NMR (Bortiatynski et al., 1996). Most

of the analyses with ^{15}N -NMR spectroscopy have thus far been confined to ^{15}N labeling studies. Using ^{15}N -labeled soil samples or melanoidins, the method is reported to have detected the presence of amine and pyrrole-type N (Stevenson, 1994; Bortiatynski et al., 1996). Recent investigations using ^{15}N -labeled aniline indicate that aniline undergoes nucleophilic reactions with the carbonyl groups of humic acids (Thorn et al., 1996).

On the other hand, very little is known yet on ^{15}N -NMR spectroscopy of the distribution of N in the various functional groups in the humic molecule. Several scientists are of the opinion that ^{15}N -NMR can be applied to analysis of N, as is the case of ^{13}C -NMR for the analysis of C. Similarly as with ^{13}C -NMR, solid-state CP-MAS ^{15}N -NMR spectroscopy is also noted to work better than the liquid state. Bortiatynski et al. (1996) are convinced that the solid-state spectra are also quantitative for measuring N in the various types of compounds in the humic molecule. However, assignments of ^{15}N -NMR signals relative to the standard nitromethane ($\delta = 0$ ppm) are given by Bortiatynski and coworkers mostly in the negative region of the chemical shift. The main signal with the strongest intensity is at -257 ppm, identified by the authors to be caused by secondary amides. Less intensive signals are noticed between -285 and -325 for NH_2 derivatives, and between -325 and -350 ppm for aliphatic amines, as illustrated in Figure 8.9. This is in contrast to Thorn et al. (1996), who show spectra of reference IHSS fulvic acid with ^{15}N signals in the positive region of chemical shifts (Figure 8.9, bottom). The dominant signal at $+121$ ppm in the reference fulvic acid sample is believed to be caused by amide-nitrogen, whereas the peak at 30 ppm shows the presence of free amino nitrogen, including amino acids and amino sugars. Indoles, pyrroles, imides, and lactam nitrogens are suspected to be the reasons for the signals between 135 and 185 ppm, with maxima at 156 , 167 , and 179 ppm. The ^{15}N -NMR spectrum of humic acid (not shown) has only one dominant peak at 120 ppm, and differs from that of fulvic acid by the absence of signals between 135 and 185 ppm.

8.5.4 Phosphorus-31 Nuclear Magnetic Resonance

Another NMR method that has recently also attracted research attention is ^{31}P -NMR spectroscopy for the characterization of P in various substances in soils. However, since the P content in humic substances is usually very low, it has not been applied to analyze the distribution of P in the humic molecule. The method has mainly been used in the past to determine the form of P extracted from soils. Several authors claim to have detected with

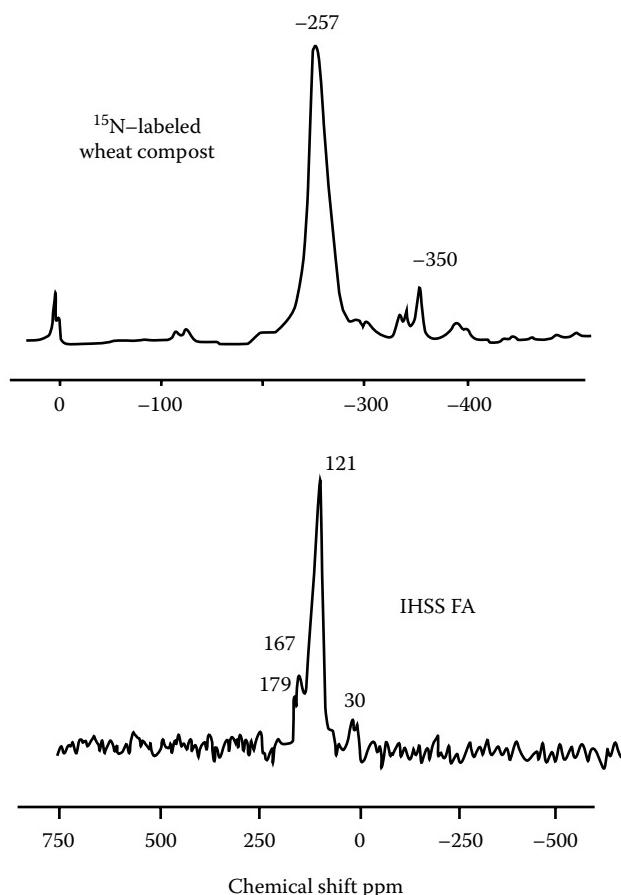


Figure 8.9 Comparison between solid-state ¹⁵N-NMR spectra of compost from ¹⁵N-labeled wheat, and “reference” IHSS fulvic acid, showing naturally occurring N at contrasting chemical shifts. (Reprinted with permission from Bortiatynski, J. M., P. G. Hatcher, and H. Knicker. NMR techniques (C, N, and H) in studies of humic substances. In: *Humic and Fulvic Acids. Isolation, Structure, and Environmental Role*, ACS Symposium Series 651, pp. 57–77. Copyright 1996 American Chemical Society; Reprinted with permission from Thorn, K. A., W. S. Goldenberg, S. J. Younger, and E. J. Weber. Covalent binding of aniline to humic substances. In: *Humic and Fulvic Acids. Isolation, Structure, and Environmental Role*, ACS Symposium Series 651, pp. 299–326. Copyright 1996 American Chemical Society.)

³¹P-NMR analysis the presence of orthophosphate ions, orthophosphate diesters, phosphate esters, and humophosphates in soil extracts. The latter is a chelated form of P, in which H_2PO_4^- , HPO_4^{2-} , and/or PO_4^{3-} ions are chelated by the carboxyl and phenolic-OH groups of the humic acid molecule. Some of the results indicate that most of the organic P in soils is present in the

form of phosphate esters, whereas smaller amounts exist as alkylphosphonates (Ogner, 1983; Newman and Tate, 1980; Glonek et al., 1970). Recently ^{31}P -NMR spectroscopy has been applied to identify forms of P chelated by humic acid from apatite, AlPO_4 , and FePO_4 minerals (Lobartini et al., 1989, 1994, 1998). A ^{31}P -NMR spectrum of the dissolution product resulting from the reaction between apatite and humic acid (at pH 9.0) is shown in Figure 8.10. As can be noticed, the main signals indicate the presence of orthophosphate diesters at -0.81 ppm , orthophosphate ions at 2.69 ppm , and humophosphates at 4.04 , and 18.31 ppm chemical shifts. The signals at 4.04 and

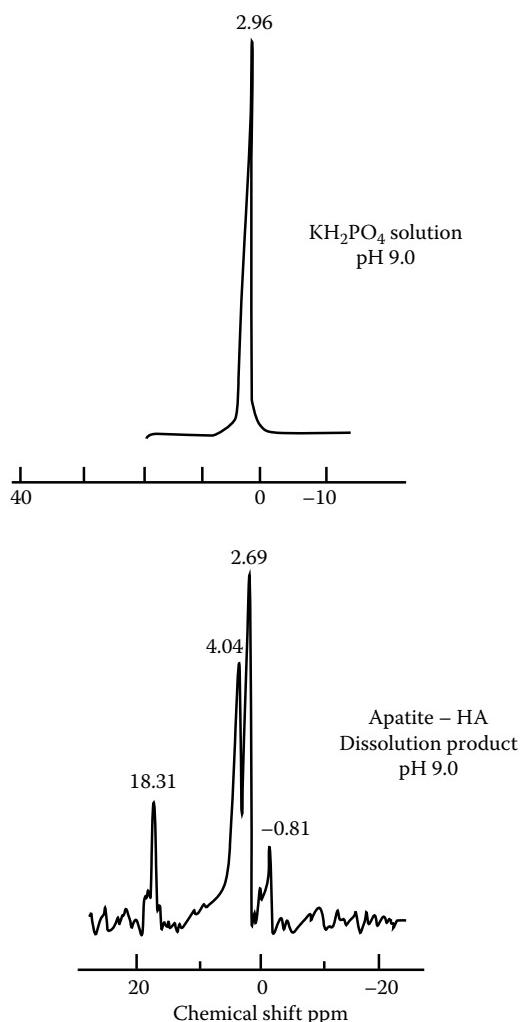


Figure 8.10 ^{31}P -NMR spectrum of pure H_2PO_4^- and a dissolution product from the reaction between apatite mineral and humic acid at pH 9.0. (From Lobartini, J. C. et al., *Comm. Soil Sci. Plant Anal.*, 25, 2355–2369, 1994.)

18.31 ppm disappear when the reactions between apatite and humic acids have been carried out at pH 5.0. At lower pH values, the functional groups, COOH and phenolic-OH, are protonated, preventing the interaction of P ions with humic acid (Lobartini et al., 1994; Tan, 1986).

8.6 Electron Microscopy of Humic Matter

The use of electron microscopy in the study of humic compounds has received considerable research attention. Two types of electron microscopy are available for this purpose, transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The advantage of TEM is that it can reach high magnification without distorting the resolution. In contrast, SEM can only be used at lower magnification for good resolution, but it can provide a three-dimensional (3D) picture that is not possible with TEM. Sample preparation is also less complex in SEM than in TEM analysis.

8.6.1 Transmission Electron Microscopy

Flaig and Beutelspacher (1951, 1954) are perhaps among the first who have tried electron microscopy for analyzing the shape and size of humic particles. Employing a transmission electron microscope, the authors above noticed that humic acids exist as very small spherical particles on the order of 10–15 nm in diameter. The spheres are frequently joined together in racemic chains. Since then the investigations carried out using TEM have yielded mixed results on the macromolecular structure of humic substances. Visser (1963) shows a structureless film of humic acid by TEM, whereas Dudas and Pawluk (1970) claim that humic acids from chernozemic soils are composed of tiny spherical particles united into spongy aggregates. Although Khan (1971) has also presented electron micrographs showing loose spongy structures for humic acids, he believes that on the basis of viscosity measurements the humic particles are in fact nonspherical. More recently, three types of fulvic acid particles have been reported from TEM investigations by Schnitzer and Kodama (1975): (1) small spheroids (1.5–2.0 nm), (2) aggregates of spheroids (20–30 nm), and (3) an amorphous material perforated by voids (50–110 nm). From electron diffraction analysis, Schnitzer and Kodama (1975) indicate that fulvic acid (pH 2.0) exhibits a crystalline structure. Since the spherical nature of the humic particle has also been mentioned in Russian literature (Orlov, 1985), many European scientists

tend to believe that at low concentrations humic acids are tiny balls. These balls coalesce at higher concentrations into sheets with a granular surface as frequently noticed by TEM. Using a replica technique, Stevenson and Schnitzer (1982) show a sequential process of coalescence of fulvic acid spheroids into aggregates with a chain-like structure in samples examined by TEM from dilute to more concentrated areas. In dilute aqueous solutions, the humic substances exist as flat, multibranched, stretched fibers or filaments, 20 to 100 nm wide, whereas at higher concentrations, these chain-like or fibrous-like structures seem to be converted into perforated sheets. Six major types of humic structures have been recognized by the authors above: small spheroids, flattened aggregates of spheroids, linear, chain-like assemblies of aggregates, flattened filaments, and perforated sheets.

8.6.2 Scanning Electron Microscopy

This method has been used extensively in the study of humic substances. Especially in Canada, Schnitzer and coworkers have applied it in studying the texture and fabric of humic and fulvic acids. As indicated above, SEM has the advantage over TEM of being capable of furnishing a 3D picture with a resolution depth of 5–10 µm. In addition, humic particle surfaces and orientation can also be shown. However, the method used is complicated, and the preparation of samples for SEM is very tedious and time consuming, hence scaring away many people from using it. Chen and Schnitzer (1976) use a freon–liquid–N preparation technique adapted from the preparation of animal tissue for surface SEM. However, the inclusion of freon gas treatment and preparing and drying samples first on glass slides or mica sheets for later transfer onto SEM specimen stubs are unnecessary. To make it simpler and faster, Tan (1985) suggests placing a drop of humic solution directly on a SEM specimen stub. Ultrarapid freezing in liquid–N and appropriate drying of the frozen specimen under a high vacuum (6.5×10^{-10} MPa) for 24 h are required for producing the proper micrographs. Failure to do so, like slow freezing in a refrigerator and slow drying in a desiccator, will yield poor resolutions or pictures showing only featureless, massive structures of humic substances (Tan, 1985).

The modified method proposed by Tan (1985) produces similar tissue-like structures for fulvic acid and humic acid as with the freon–liquid–N method on mica strips. Except for better 3D depth, the structures revealed by SEM are also not much different from those produced by TEM. As can be noticed in Figure 8.11, fulvic acid at pH 3.0 exhibits a fiber-like structure. The fulvic

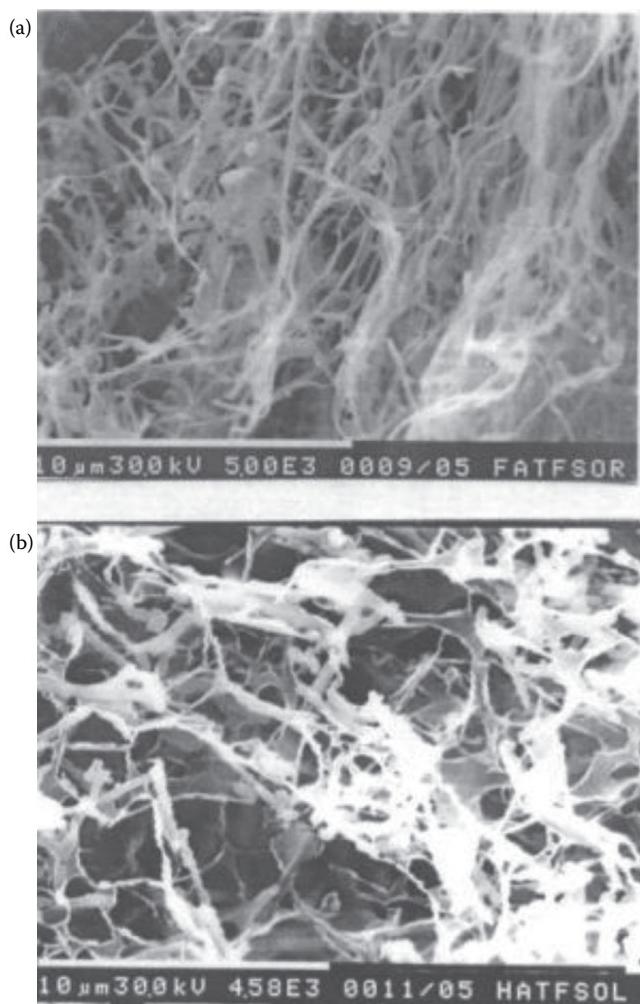


Figure 8.11 Scanning electron micrographs of (a) fulvic acid and (b) humic acid, extracted from a Tifton soil (ultisols) in Georgia, United States, showing the characteristic fiber structures. Magnification: white bar = 10 μm .

acid fibers ranged in thickness from 0.2 to 5 μm , and some soils possess fulvic acids with thick fibers, whereas other soils contain fulvic acids with thin fibers. The humic acid (pH 7.0) structure is almost similar but tends to be more like shredded sheets in appearance. The observations above for slight differences in fulvic acid and humic acid structure can also be noticed for aquatic humic substances. The micrographs in Figure 8.12 show aquatic fulvic acid to be characterized by thin fibers, whereas the humic acid counterpart has a structure composed of relatively slightly thicker fibers.

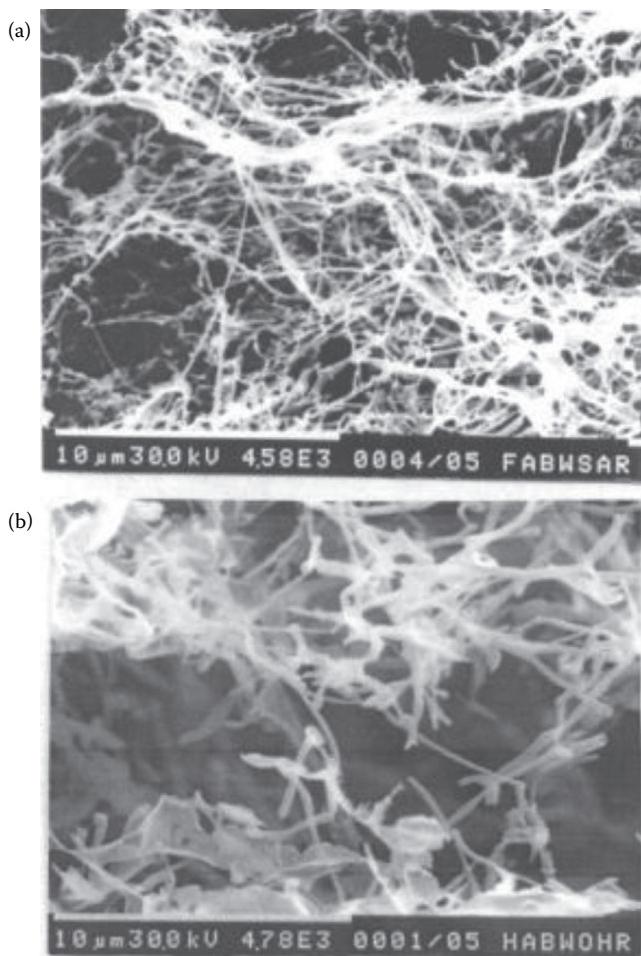


Figure 8.12 Scanning electron micrographs of aquatic (a) fulvic acid and (b) humic acid, extracted from black water of the Satilla and Ohoopee rivers, respectively, in southeast Georgia, United States, showing similar fibrous-like structures. Magnification: white bar = 10 μ m.

Chen and Schnitzer (1976) believe that the differences in humic structure are the effect of pH. Protonated fulvic acid (pH 2–3) is reported by the authors to have an open structure formed from elongated fibers with rounded tips. This structure is noted to change into a sponge-like structure at pH 7.0, and at pH 9.0 it becomes a homogeneous sheet in which grains are visible. However, the effect of pH on humic acid structure cannot be supported or reproduced by the current author. Perforated sheet structures have been noticed by the present author in humic acids at pH 3.0, as can be seen in Figure 8.13. The humic acid solution was prepared and purified by

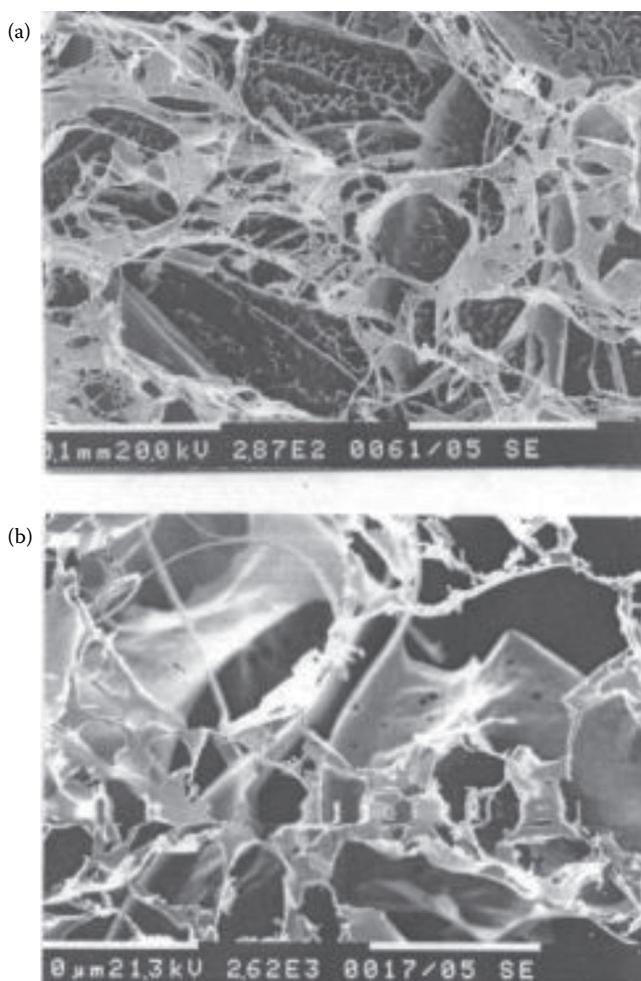


Figure 8.13 Scanning electron micrographs of humic acids (pH 3.0) extracted from (a) a Cecil soil (ultisols) in Georgia, United States, (magnification: white bar = 0.1 mm), and (b) an andosol in Indonesia (magnification: white bar = 10 μ m), showing perforated sheet, identified now to be humo-nanotube membranes structures.

thorough leaching through an H-saturated cation exchanger column according to the method of Lakatos et al. (1977) as described earlier. The purified humic acid solution flowing from the cation exchange column is highly protonated and has a pH of 3.0 (Tan, 1996), and is used directly for SEM analysis. From the discussion above, it appears that, in general, only two major types of structures can be distinguished, i.e., (1) stretched fibers and (2) perforated shredded sheets, as illustrated by the examples in Figures 8.11 and 8.12, and Figure 8.13, respectively.

The other types of structures recognized by Schnitzer and coworkers in Canada can be considered intergrades between the two major types above. Although soil pH has some effect, the current author believes that the concentration has perhaps a more dominant role in affecting structural changes. With increased humification, more humic substances are being produced, and in a more concentrated (crowded) condition several of the humic fibers are bundled together and forced to fuse or coalesce into thin, flat strands, which eventually become perforated or shredded sheets with a continued concentration increase of humic substances.

The fiber- or tissue-like structures are apparently characteristic of the humic substances, regardless of origin or method of determination. As indicated earlier, both TEM and SEM yield similar structures, suggesting reproducibility of results. The humic substances used for TEM and SEM analyses by the Canadian scientists have been extracted from spodosols, acid soils of the humid temperate and cool regions of North America. On the other hand, the humic substances used for SEM by the current author originate from ultisols, acid soils of the humid warm temperate to subtropical region of the southeastern United States. Yet the Canadian spodosol humic substances exhibit similar fibrous structures as the U.S. ultisol humic compounds. By comparison, no differences have also been noticed for the structures of aquatic humic matter. The question is raised now whether artifacts could have produced such reproducible structures, or whether all these confirm the possibility for the presence of real humic and fulvic acids in nature.

8.6.2.1 Humo–Nanotube Analysis by SEM

With the recent introduction of the supramolecular and especially the humo–nanotube concept in humic acid chemistry (see Chapter 3, Section 3.7), new detailed structural arrangements are discovered, requiring the present author to add new types and/or revise some of the interpretations of the older observations. By adjusting toward bias to the more modern concepts, the older interpretations of fibrous humic structures have to be changed and reidentified as carbon nanotube structures and carbon nanotube bundles. Evidence for the presence of both these structures has been presented earlier by electron microscopy in Figure 3.3, revealing the characteristic nanogrid pattern, like a fishnet, of carbon nanotubes, and the distinctive tubular assembly of nanotube bundles, respectively. The so-called fishnet structures seem to be arranged further into designs similar to the cells of honeycombs (see Figure 3.4). The micrograph and photograph

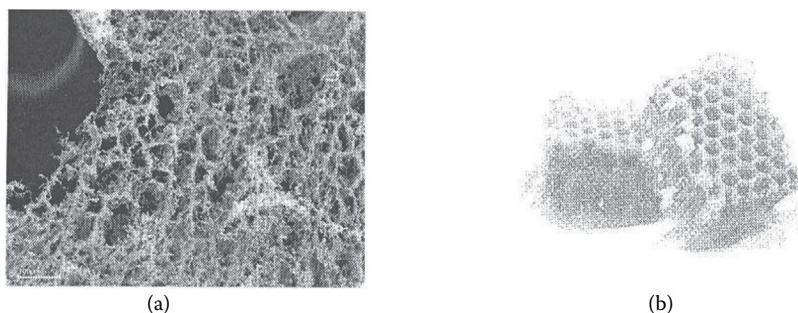


Figure 8.14 (a) Scanning electron micrograph (magnification, see Figure 3.4) of a humic acid sample extracted from lignite with a humo-nanotube membrane structural assembly. (b) Photograph of a beehive honeycomb-cell pattern.

provided in Figure 8.14 for comparison confirm the very close resemblance of a humo-nanotube structural assembly with a honeycomb-cell pattern as exhibited in beehives. These types of structures, called earlier humo-nanotube membranes, have apparently been overlooked or identified only as membranes in the older investigations. However, carbon nanotubes and humo-nanotube membranes seem to be more common than expected. As can be noticed, for example, in Figure 8.13a, they occur all over in the micrograph as bits and pieces, sometimes damaged perhaps due to the process of handling during preparation of the sample for analysis. A humo-nanotube membrane is clearly visible in the upper right corner of the micrograph exhibiting a slightly damaged honeycomb design, whereas—situated at 8 o'clock below it—a carbon nanotube sheet is located showing the characteristic fish net pattern. At the top middle section of the micrograph, another humo-nanotube membrane can be noticed with a slightly more damaged pattern, and so on toward the bottom of micrograph A in Figure 8.13. These types of humo-nanotube structures are not operational or fake features, but occur naturally, since they are easily reproduced by SEM with other humic samples as long as the proper protocol of sample preparation is maintained. The micrographs presented in Figure 3.4 testify for the ease and dependability of the rapid procedure as published by Tan (1985, 1996). By focusing at suggested desired spots, scientists at the University of Georgia succeed with a different but more modern SEM instrument in singling out the respective humo-nanotube membrane structures as shown in the figure mentioned above (Tan, 2011a, b).

Chapter 9

Electrochemical Properties of Humic Matter

9.1 Origin and Types of Electric Charges

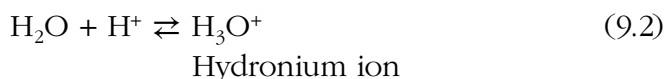
9.1.1 Amphoteric Concept of Humic Substances

Humic and fulvic acids are considered *amphoteric* compounds, but Stevenson (1994) assumes them to be weak acids. With the recent introduction of the supramolecular, micellar, and nanotube concepts, they are also defined as associations containing submicroscopic aggregates of *amphiphilic* molecules. Hence, at the current stage of knowledge, it is perhaps better to view humic substances as an association that exhibits both amphoteric and amphiphilic features. Although both these features show close relations with electrochemistry, they are defined differently with *amphotericism* conceptually based more on models of chemical substances that can behave as acids as well as bases, whereas on the other hand the idea of *amphiphiles* consists of chemical compounds with both hydrophilic and hydrophobic properties (Tan, 2011). Since amphotericism deals closely with acid–base chemistry, it is this property that is directly responsible more for the development of electrical charge characteristics of humic substances. The term *amphiprotic* is also used to indicate the property, whereas the chemical species exhibiting both the acidic and basic characteristics is called an *ampholyte*. All these terms find their roots in the Greek word *amphi*, meaning “both.” At present, three models are commonly used in describing acid–base relations in soil chemistry, the *Arrhenius*, *Brønsted–Lowry*, and *Lewis* concepts (Tan, 2011). The

fact that humic substances can act as acids or as bases makes the application of the Arrhenius concept rather untenable and, in the present author's opinion, the last two concepts above seem to be more appropriate to describe adequately the reactions involving humic substances as amphotytes. The theory of the Brønsted–Lowry model is perhaps more applicable in relation to ion exchange reactions, whereas the Lewis theory is more adaptable for use with complex reactions and chelation processes exhibited by humic substances. The creation of both negative and positive charges due to amphotermism is an electrochemical property regarded as a distinctive feature of humic substances. The negative charges have usually been studied more intensively and, consequently, are better known than the positive charges. All these charges are developed by the ionization and dissociation of various functional groups of humic compounds. The creation of different types of charges and their relation with acid–base reactions will be explained in some detail in the following sections below.

9.1.1.1 Brønsted–Lowry Acid–Base Concept

The Brønsted–Lowry theory, also known as the *proton concept* in electrochemistry, defines acids as proton donors, whereas bases are proton acceptors (Tan, 2011). Therefore, an amphotyte can either donate or accept protons. Water and carbonic acids are the most common examples of simple amphotytes, and as shown in the reactions below, water is capable of donating and accepting protons:



Reaction 9.1 is a dissociation reaction, in which water is dissociated into a hydrogen ion and a hydroxyl ion. The Arrhenius definition of acids limits them to compounds containing hydrogen ions only. On the other hand, bases are defined as compounds containing hydroxyl ions only. The above reaction indicates that water is both an acid as well as a base; hence, it is amphoteric. The hydrogen ion is, in fact, a bare proton; therefore, the terms *hydrogen ion* and *proton* are often used interchangeably. In reaction

9.1, water can be considered “donating” a proton and is then an acid by the Brønsted–Lowry definition. On the other hand, in reaction 9.2, water is accepting a proton with the consequent formation of a hydronium ion, since free hydrogen ions are not present in the soil solution. Water is then, by definition of the proton concept, also a base. The reactions above are, of course, pH dependent with reaction 9.1 occurring at high soil pH whereas reaction 9.2 takes place at low soil pH. At a certain point in between, the amount of negative charges equals that of the positive charges and the net surface charge is then zero. The pH where this occurs is called the *PZC* (*point zero charge*) or *ZPC* (*zero point charge*) in soil chemistry. In biochemistry, it is the *isoelectric point*.

When the dissociation (deprotonation) and protonation processes described above occur with the carboxyl and phenolic-OH groups, the two major functional groups of humic acids, similar negative and positive charges are created. These electrochemical charges are responsible for ion exchange reactions. The total negative charge developed by deprotonation of both the carboxyl and phenolic-OH groups is also called the *total acidity*, and is usually taken as the *cation capacity* of humic substances.

9.1.1.2 Lewis Acid–Base Concept

According to this concept, an acid is a compound that can accept an electron pair and a base is then a substance that can donate an electron pair (Tan, 2011). Therefore, any chemical species that has an unshared pair of electrons to donate is a base, whereas any compound that accepts a pair of electrons is an acid. This is called also the *electron-pair concept*, and a simplified reaction, involving electron-pair donation and sharing, is given below as an illustration:



In the reaction above, the H^+ ion accepts an electron pair and, by the Lewis definition, is then an acid. The bond is called a *covalent bond*. The OH^- ion is the electron-pair donor and is then the base. This is the reaction noticed to occur with humic substances. According to the electron-pair concept, humic acid is a base and seems to function only as an electron-pair donor through dissociation of the H^+ ion from the phenolic-OH groups. This enables the humic molecule to donate the exposed electron pair to other

elements or substances, resulting in the formation of covalent-bonded coordinated complex compounds. The reaction is called *complex* or *complexation reaction*. When conditions are favorable for the carboxyl groups to additionally function as electron-pair donors, they will form together with the phenolic-OH groups a multiple electron-pair donor system. Such a system is capable of conducting reaction processes known by the name of *chelation*, which is characterized by a peculiar ring type of chelate bonding (Greek *chele* = lobster claw) of substances. The humic molecule is called the *organic ligand*. More details will be provided in Section 9.5, whereas the principles of complex formation and chelation are examined by Tan (2011).

9.1.2 Negative Charges

As indicated in the preceding sections and in particular from the proton concept in Section 9.1.1.1, the negative charges are attributed to dissociation of protons from the functional groups in the humic molecule. In chemistry a great number of chemical groups are defined as functional groups. Fifty types have notably been recognized, from the simple methane, CH_4 , and amino group, NH_2 , to the more complicated peroxide, C-O-OH , and to the highly complex polycyclic aromatic hydrocarbon groups. In humic acid chemistry, the chemical groups that contribute most to the surface charges and reactivity of humic molecules are called *functional groups*. The two most important functional groups in this respect are the carboxyl, COOH , and phenolic-OH groups. The *alcoholic hydroxyl* groups have been examined in Chapter 7, Section 7.2.1, to be of minor importance in the creation of electronegative charges, which is supported by current ideas of interaction reactions with metals (Coles and Yong, 2006). In general, the two functional groups indicated above control the electrochemical behavior of humic matter and are the main reasons for adsorption, cation exchange, complex reactions, and chelation reactions. As discussed in Chapter 7, Section 7.2.1.1, the COOH contents tend to be higher than the amount of phenolic-OH groups in the humic molecules. It can also be noted from the literature data that different methods of determination may also have affected the large variations found in results on functional group composition, contents, and total acidity of humic substances. This is also evident from the more current data of Ritchie and Purdue (2003), showing substantially higher contents of COOH than phenolic-OH groups. The extensive data are posted also in the website by the authors above as an International Humic Substances Society

(IHSS) report (<http://www.humicsubstances.org/acidity.html>, accessed June 8, 2013). By using the NaOH determination method, instead of the common Ca-acetate method, the higher contents of COOH groups seem to be greatly increased. Some of the very extensive amounts of the original data of Ritchie and Purdue (2003) are converted into averages by the present author and presented in Table 9.1 to show the enhanced differences between COOH and phenolic-OH functional group contents. Especially, the very high COOH contents of fulvic acids, reported to range from 11.3 to 13.3 meq/g, seem to support somewhat the data published by Schnitzer (1977) and may tend to be controversial in nature, an issue examined in more detail in Section 9.1.2.3. These high COOH group values will yield with the phenolic-OH contents, total acidity values of well above 1000 meq/100 g. Since the total acidity reflects negative charges equivalent to the cation exchange capacities of humic substances, values of exchange capacities >1000 meq/100 g—two in Table 9.1 >1500 meq/100 g—are normally considered excessively high. Until more data can become available, it seems the NaOH method is biased toward yielding high COOH group concentrations in the determination of functional group contents in humic acids.

Table 9.1 Data Selected from the IHSS Report on Functional Group Content, Recalculated by the Present Author into Average Values ([meq/g]·k)

| | <i>Carboxyl (COOH)</i> | <i>Phenolic-OH</i> | <i>Total Acidity</i> |
|--|------------------------|--------------------|----------------------|
| Suwannee River, Georgia, USA | | | |
| HA | 9.4 | 4.0 | 13.4 |
| FA | 11.3 | 2.9 | 14.2 |
| Aquic Argiudolls (Elliott soil)^a | | | |
| HA | 8.3 | 1.9 | 10.2 |
| FA | 13.2 | 2.3 | 15.5 |
| Pahokee Peat | | | |
| HA | 9.0 | 1.9 | 10.9 |
| FA | 13.3 | 2.3 | 15.6 |

^a Elliott soil is correlated by the present author into its higher U.S. Soil Taxonomy category placement as Aquic Argiudoll, a mollisol under poorly drained conditions.

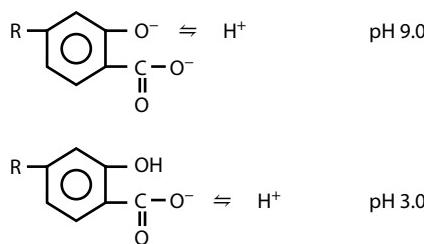


Figure 9.1 Development of variable charges in a humic molecule by dissociation of protons from carboxyl groups at pH 3.0 and from phenolic-OH groups at pH 9.0.

The carboxyl, COOH, groups start to dissociate their protons at pH 3.0 (Posner, 1964) and the humic molecule becomes negatively charged (Figure 9.1). When this charge reaches a maximum value, only half of the amount is carried by the carboxyl groups as predicted by the laws of equilibrium discussed in Section 9.1.2.1 below. At pH <3.0, the charge is perhaps very small or even zero. At pH 9.0, the phenolic-OH groups also dissociate their protons, and the humic molecule attains a high negative charge.

Since the development of the negative charge is pH dependent, this charge is called *pH-dependent* charge or *variable charge* (Tan, 1998). As indicated above, this charge is expected to be small at low pH, but is large at high pH, which corresponds to low cation exchange capacity (CEC) at low pH and high CEC at high pH. According to the theory of CEC, the negative charge will eventually reach a maximum value at pH 8.2. This will be explained further in Section 9.4 on CEC.

9.1.2.1 Significance of the Henderson–Hasselbalch Equation

Generally, the ionization of amphoteric compounds can be studied by using the law of equilibrium or concept of pK values. By assuming that the dissociation of humic acid (HA) proceeds as follows:



then, the equilibrium constant K of the reaction above is given by

$$K = \frac{(\text{H}^+)(\text{A}^-)}{(\text{HA})} \quad (9.5)$$

By converting into $-\log$, Equation 9.5 changes into

$$-\log K = -\log \frac{(H^+)(A^-)}{(HA)} \quad (9.6)$$

$$pK = -\log(H^+) + \left[-\log \frac{(A^-)}{(HA)} \right] \quad (9.7)$$

$$pK = pH - \log \frac{(A^-)}{(HA)} \quad (9.8)$$

Equation 9.8 is the famous Henderson–Hasselbalch equation. It describes the ionization process of amphoteric compounds; hence, it applies to ionization of humic acids. When ionization has proceeded to the point where the concentration or activity of $(A^-) = (HA)$, the equation changes into

$$pK = pH \quad (9.9)$$

This pK is often referred to as pK_1 and pK_2 , or ionization constants for the dissociation of COOH and phenolic-OH groups, respectively. In titration analysis, the condition defined by Equation 9.9 usually occurs at half-neutralization. The pK_1 value is considered to be an intrinsic value and should apply to all the acidic or carboxyl groups in the humic molecule. As indicated above, half of the COOH functional group contents are then deprotonated when $pH = pK$, which Posner (1964) expects to occur at pH 3.0. An illustration of the dissociation process is given in Figure 9.1. Consequently the humic molecule surface contains half of the amount of charges carried by the COOH groups. The negative charges contributed by the COOH groups will increase with an increase of soil pH. However, the conditions for $pH = pK$ to occur with the phenolic-OH groups can take place only when the surrounding soil solution is very low in protons. This is postulated by Posner (1964) to occur at pH 9.0 (see also Figure 9.1). However, some believe that the deprotonation of COOH groups discussed above may occur at higher pK values. Wilson and Kinney (1977) claim dissociation of carboxyl functional groups to take place at pK_1 4.0, which is supported by $\log K_1$ values presented by Ritchie and Purdue (2003) ranging from 3.67 to 4.59. Ritchie and Purdue also state pK_2 values ranging from 9.52 to 10.74 for the

phenolic-OH groups to dissociate, hence at even higher pK values than the pK_2 in Figure 9.1, when half-neutralization is attained.

9.1.2.2 Use of pK_a in Determining Negative Charges

In soil chemistry, the magnitude of the ionization constant K or the pK value is used as an indication for the degree of ionization. The symbol K is often written as K_a , to indicate an ionization reaction. As can be noticed from Equation 9.5, the higher the value of the ionization constant K, the larger will be the value of $(H^+)(A^-)$ and the smaller the amount of (HA). This means that at high K_a values (or low pK_a values), large amounts of (HA) are ionized into H^+ and A^- ions. Ionization is less at low K_a or high pK_a values. In pure chemistry, substances characterized by high ionization constants (or low pK_a s) are called *strong acids*, in contrast to those with low ionization constants (or high pK_a s), which are considered *weak acids*. Conforming to the Henderson–Hasselbalch concept, ionization amounts to only 50% at $pH = pK$ ($= pK_a$). Stevenson (1994) assumes that at one pH unit above the pK_a , the acidic groups of the humic molecule will be 90% ionized, whereas at two pH units above the pK_a , the acidic groups are estimated to be 99% ionized. In contrast, at one pH unit below the pK_a , the functional group is only 10% ionized, whereas at two pH units below the pK_a , ionization amounts only to 1%, where the negative charge is close to zero. In support of the predictions above on the extent of variable negative charges created by COOH groups at high and low pH values, the following calculations are presented by the present author. As examined above, substantial amounts of negative charges are expected to be present at $pK_a = pH$, which will increase in magnitude and reach maximum values at two pH units above this pK_a . The two examples of computations below with dissociation of COOH groups provide statistical evidence for 50% ionization at $pH = pK_a$ and the validity of predictions for pH above or below pK_a .

According to Equation 9.8:

$$pK - pH = -\log (COO^-)/(COOH) \quad (9.10)$$

At $pH = pK$ ($= pK_a$) = 3.0

$$0 = -\log (COO^-)/(COOH) \rightarrow (COO^-)/(COOH) = 1$$

$$(COO^-) + (COOH) = 100\% \rightarrow (COOH) = (100 - COO^-)$$

Therefore, $(\text{COO}^-)/(100 - \text{COO}^-) = 1$

$$2(\text{COO}^-) = 100$$

$$(\text{COO}^-) = 100/2 = 50\%$$

At pH = 5.0 and pK ($= \text{pK}_a$) = 3.0

$$3.0 - 5.0 = -\log (\text{COO}^-)/(\text{COOH})$$

$$2.0 = \log (\text{COO}^-)/(\text{COOH}) \rightarrow (\text{COO}^-)/(\text{COOH}) = 10^2$$

$$(\text{COO}^-) + (\text{COOH}) = 100\% \rightarrow (\text{COOH}) = (100 - \text{COO}^-)$$

Hence, $(\text{COO}^-)/(100 - \text{COO}^-) = 100$

$$(\text{COO}^-) = 100 (100 - \text{COO}^-)$$

$$101 (\text{COO}^-) = 10,000 \rightarrow (\text{COO}^-) = 10,000/101 = 99.0\%$$

9.1.2.3 Issue of COOH Groups

The level or degree of electronegative charges is not only affected by the degree of ionization of the active functional groups, but it also depends on the concentration or relative distribution of these groups in the humic molecule. The larger the concentrations of the functional groups, the higher will be the negative charges of the humic molecule. The relative distribution of these functional groups is noticed to vary widely from soil to soil, and a considerable variation is also present for humic matter within similar soil groups. As discussed in Chapter 7, the opinion is that fulvic acids are generally higher in carboxyl group contents than humic acids. Schnitzer (1977) and, more recently, Ritchie and Purdue (2003) have reported even more dramatically larger differences in carboxyl group contents between fulvic and humic acids than shown in Table 7.5 of Chapter 7 and Table 9.1 of this chapter. The carboxyl contents in fulvic acids are shown by Schnitzer to range between 5.20 and 11.20 me/g as compared with a range of 1.50 and 5.70 me/g for humic acids, extracted from soils worldwide. However, the above is contradicted by the studies conducted by Tsutsuki and Kuwatsuka (1978), involving a large number of humic acids, extracted also from a wide variety

of soils. Their results indicate that the COOH content increases whereas the phenolic-OH group content decreases during the humification process. This suggests that humic acid, the product of advanced humification, would be higher in COOH content than fulvic acid, the substance formed at the start of humification. This idea agrees fairly well with that of Coles and Young (2006), who indicate that during formation of humic acids from fulvic acids, carbonyl ($C=O$) groups increase in amounts that tend to be transformed by oxidation into carboxyl (COOH) groups during further humification. It is, however, in sharp contrast with Stevenson's (1994) theory on diagenetic transformation of humic acid into fulvic acid, as discussed earlier. The controversial opinions above make the issue of COOH content confusing and leave us wondering what or whom to believe. Regardless of all the reservations offered above, the latter do not indicate that Schnitzer's, Ritchie and Purdue's, or Stevenson's data are incorrect; they only mean that one has to use caution in accepting the facts on COOH content in humic substances. The latter finds its origin in the difficulties encountered in the analysis of functional groups. As discussed earlier, different analytical methods may yield different results, while the exact measurement for accounting for the acidic groups is also subject to many errors.

Both carboxyl and phenolic-OH groups generally contribute to the development of negative charges; however, the opinion exists that the carboxyl groups are the most important in the formation of negative charges. This is perhaps true and can be explained by applying the Henderson–Hasselbalch concept. If we can assume that the COOH groups dissociate their protons at pH 3.0 as postulated by Posner (1964), and at this condition $pH = pK_1$, then 99% ionization will be reached at pH 5.0, as calculated above with Equation 9.10. This pH of 5.0 is a “normal” pH value in most acidic soils generally considered productive for agricultural operations, especially forestry. In contrast, the phenolic-OH groups will be dissociating their protons at pH 9.0, a pH value seldom occurring in agricultural soils. If the assumption is made again that at this condition, $pH = pK_2$, then 99% ionization will be reached at pH 11.0, a pH value too high to be agriculturally productive in even the best aridisols. A possibility is that the pH value of 9.0, as postulated by Posner (1964) for the dissociation of phenolic-OH groups, is far too high and valid only for laboratory conditions but not for the natural soil condition. Chelation and complex reactions are noticed to take place at pH 4.0–8.0 in natural soil environments. Apparently, more research has to be conducted to confirm or revise the exact pH for the dissociation of especially phenolic-OH groups in natural soils.

9.1.2.4 Significance of Total Acidity in Negative Charges

As explained in Chapter 7, the sum of the carboxyl and phenolic-OH groups is defined as the total acidity; hence, this property should also reflect the level of negative charges of humic substances. A high total acidity value is then indicative of the presence of high negative charges. A low total acidity value, in turn, points to the presence of low negative charges. Since fulvic acids exhibit higher total acidity values than humic acids, they are expected to be higher in negative charges than humic acids. However, this does not necessarily mean that fulvic acid has a higher chemical activity than humic acid. Results of studies on chelation and complexation analyses indicate that metal chelation by humic acids appears to be more effective than that by fulvic acids. The amounts of metals chelated by humic acids are always higher than those chelated by fulvic acids (Tan, 1978; Lobartini et al., 1994). Most people assume this to be caused by the differences in sizes and complexity between the two types of humic substances (Stevenson, 1994). The substantially larger molecules and the more complex structures of humic acids are accepted to be the reasons for more binding sites and higher binding capacity in contrast to fulvic acids, which are smaller and less complex. In this respect, the following hypothesis is added by the current author for further contemplation. In the preceding sections, fulvic acids have been described as possessing higher COOH contents than humic acids. Carboxyl groups, in general, exhibit their chemical activities through their acidic (H^+) reactions only. They are effective in cation exchange reactions, but they display little or no chelation, although some complex reactions may be present (Tan, 1986). Acetic acids and formic acids are compounds in this category since their acidic characteristics are attributed to the presence of only COOH groups in their molecules. On the other hand, humic acids exhibit acidic characteristic attributed to the presence of COOH groups and especially substantial amounts of phenolic-OH groups. Because of these two groups, humic acids have the advantage over fulvic acids by being able to exert both an acidic (H^+) reaction and a strong or large interaction effect. The interactions can be in the form of electrostatic attraction, complex formation or chelation, and water bridging, as illustrated in Figure 9.2. By virtue of the higher phenolic-OH group content, chelation is then substantially higher by humic acids than fulvic acids. Hence, the lower content of phenolic-OH groups in fulvic acids (see Chapter 7) is perhaps an additional reason for their lower chelation capacities. In summary, the conclusion can be drawn that a high total acidity, generated by high COOH and low phenolic-OH

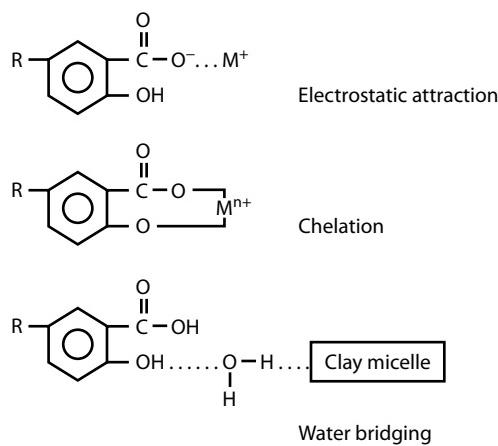


Figure 9.2 Adsorption or electrostatic attraction by humic acid (top), complex or chelation reaction (middle), and water bridging or coadsorption (bottom). M^{n+} = cation with charge n^+ ; R = remainder of the humic acid molecule.

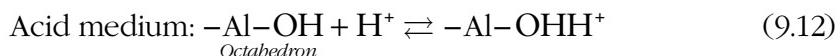
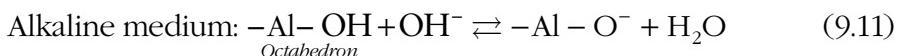
group contents, will be less effective in chelation and complexation reactions than a total acidity caused by the presence of lower carboxyl contents but in combination with high amounts of phenolic-OH groups.

9.1.3 Positive Charges

The positive charges are caused by the presence of amino, NH_2 , groups. Protonation of amino groups will create positive charges (Tan, 2011). By comparison with the oxygen-containing functional groups, the concentration of amino groups in humic substances is often believed to be relatively small. This is perhaps one of the reasons why the positive charges of humic substances are considered to be only of minor importance. However, the N contents of humic matter are substantial and do not confirm the opinions above. Considerable amounts of NH_2 groups must be present especially to account for the substantially high contents of N in humic acids. It is perhaps the inability of today's techniques in determining NH_2 groups in humic substances that have created a misconception of low amino group contents. Although the nitrous oxide method, a standard method for analysis of free NH_2 groups in proteins, shows 30% of the humic-N to be present as amino groups, the analysis is subject to many errors due to interference by lignin and phenolic groups in the humic molecule (Stevenson, 1994). Other scientists have also shown mixed results

in detecting measurable amounts of amino groups in humic substances (Sowden, 1957; Sowden and Parker, 1953). Because of the uncertainty in obtaining reliable results, the issues of NH₂ group contents and positive charges in humic matter are usually ignored. Protonation of amino groups, as taking place in zwitterions of amino acid compounds, lacks proper attention in humic acid research.

In clay mineralogy, it is noted that positive charges can also be created on mineral surfaces by protonation of exposed OH groups. Not only can protons be dissociated from these OH groups, but the latter can also adsorb and gain protons (Tan, 1998). This process of protonation is important only in a strongly acidic condition. The reactions for dissociation and protonation of exposed OH groups in clay mineralogy can be summarized as follows:



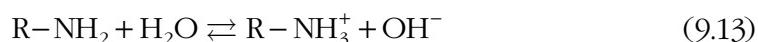
Humic substances are known to contain substantial amounts of OH groups, although, of course not associated as octahedral Al-OH groups. They are, in fact, present in the aromatic core as phenolic-OH groups, as well as on the aliphatic C-chain of the humic molecule as alcoholic-OH groups (see Chapter 7), and most of them, if not all, are located in exposed positions. Since they also react as weak acids, it is perhaps conceivable that these OH groups can also behave similarly as in reactions 9.11 and 9.12. Phenolic-OH groups have been examined above to dissociate their protons in an alkaline medium, which is considered one of the reasons for the development of variable negative charges in the humic molecule. However, how they behave in an acidic medium is another question. It has been predicted earlier by calculations that at two pH units below the pK₂ value, the phenolic-OH group is 99.0% protonated or practically nondissociated; hence, this group is essentially neutral. Although positive charges are developed on clay minerals at pH values below their ZPC, it is still a very big question whether at pH values below the “isoelectric point” of the phenolic-OH group above (e.g., pH 6–7), the acidic condition can induce protonation of phenolic-OHs. The soil pH is then between 6 and 7, which is the most

advantageous or the proper condition for plant growth. However, the creation of such a positive charge may also reduce the effect of the negative charge developed by the carboxyl group, creating another issue for the possibility of the humic molecule becoming a “zwitterion.” The latter has been established for amino acids, whereas clay minerals are known to be negatively charged on planar surfaces but positively charged on broken edge surfaces. On the other hand, humic acids have been defined earlier as amphoteric compounds, substances that behave as acids as well as bases. No direct information is available to refute or support all these assumptions with humic substances, although their cation exchange and complex reactions seem to point to these directions by decreasing substantially with a decrease in soil pH.

The other possibility, protonation of the carboxyl functional groups of humic substances, seems to be of academic importance only. Owing to their possession of C-OH radicals, these carboxyls are also capable of accepting extra protons, but then only at extremely low pH values. From similar calculations, as illustrated above for Equation 9.10, it can be shown that these COOH groups are expected to exist for 99.0% in protonated or undissociated forms at two pH units below $\text{pH} = \text{pK}_a = 3.0$. At a soil pH of 1.0, the COOH groups are practically electrically neutral and a further decrease is required in soil pH for their OH-radicals to take extra protons creating the positive charges. Unfortunately, at this extremely low soil pH, the soil conditions are practically deplorably poor or toxic for any type of agricultural operations.

9.1.3.1 Protonation of Amino Groups

The difficulty with protonation of amino groups is that the process can only occur in an acidic condition when soil pH is below the pK_a value of humic acids. The rules in basic soil chemistry indicate that amino groups will be protonated, hence carry positive charges, when $\text{pH} < \text{pK}_a$, or in other words in acid soils only, a condition for providing the required large amounts of H^+ ions. The amino groups are neutral or carry no charges in basic soils, or when $\text{pH} > \text{pK}_a$. The reaction of the amino group is, in fact, governed by a constant called pK_b , which is related to the pK_a as explained below. Protonation of an amino group can be illustrated by the following reaction:



The equilibrium constant K of the reaction above is

$$K_b = \frac{(R - NH_3^+)(OH^-)}{(R - NH_2)(H_2O)} \quad (9.14)$$

At standard conditions, the activity of water is unity; hence

$$K_b = \frac{(R - NH_3^+)(OH^-)}{(R - NH_2)} \quad (9.15)$$

Multiplying by $-\log$ gives

$$-\log K_b = -\log \frac{(R - NH_3^+)(OH^-)}{(R - NH_2)} \quad (9.16)$$

$$-\log K_b = -\log(OH^-) - \log \frac{(R - NH_3^+)}{(R - NH_2)} \quad (9.17)$$

$$pK_b = pOH - \log \frac{(R - NH_3^+)}{(R - NH_2)} \quad (9.18)$$

When the activity of $(R - NH_3^+) = (R - NH_2)$:

$$pK_b = pOH \quad (9.19)$$

Since $pH + pOH = 14$, and at half neutralization $pK_a = pH$, hence

$$pK_a = 14 - pK_b \quad (9.20)$$

In contrast to the concept of ionization of acidic groups (COOH groups) as explained earlier, the amount of amino groups protonated is now expected to be less at pH values above the pK_a , as defined now by Equation 9.18, but more at pH values below the pK_a . When $pH = pK_a$, conforming to the Henderson–Hasselbalch concept, only 50% of the amino groups are protonated. However, at one pH unit above the pK_a , protonation of amino

groups amounts only to 10%, whereas at one pH unit below the pK_a 90% of the amino groups are protonated (Stevenson, 1994). High positive charges are therefore expected to be present when $pH < pK_a$, whereas no positive charges or only low positive charges are present at $pH > pK_a$.

9.2 Surface Charge Density

In soil chemistry, the negative charges created by soil colloids are theoretically point charges. However, for practical reasons, these charges are considered evenly distributed over the colloidal surface. The magnitude of these charges is then usually expressed in terms of amount of charges per unit area. The latter is called *surface charge density*, σ_s , which can be formulated as follows:

$$\sigma_s = e/S \quad (9.21)$$

in which σ_s = surface charge density in $\text{esu}/\text{m}\mu^2$ ($1 \text{ m}\mu^2 = 100 \text{ \AA}^2$), e = number of charges per unit formula, and S = specific surface (Fripiat, 1965).

However, since the total charges on colloidal surfaces are, in fact, the contributions of permanent and variable charges, the following relationship exists:

$$\sigma_s = \sigma_p + \sigma_v \quad (9.22)$$

where σ_s = surface charge density in esu/cm^2 (esu = electrostatic unit and $1 \text{ esu} = 300 \text{ V}$), σ_p = surface charge density due to permanent charges, and σ_v = surface charge density due to variable charges.

The value of σ_p is constant but the value of σ_v is variable. Since permanent charges in humic matter are usually small and can be neglected, the following relationship is assumed to be valid for humic substances:

$$\sigma_s = \sigma_v \quad (9.23)$$

in which σ_v customarily can be calculated using the Gouy–Chapman equation as follows:

$$\sigma_v = \sqrt{[(2\eta\epsilon kT)/\pi]} \sinh(ze\psi)/(2kT) \quad (9.24)$$

in which σ_v = variable surface charge density in esu/cm², η = electrolyte concentration in numbers of ion/cm³, ϵ or D = dielectric constant of the medium, k = Boltzmann constant in erg/ion degree, T = absolute temperature in degrees Kelvin, π = a constant = 3.14, z = valence, e = electron charge in esu, and ψ = surface potential in statvolt.

The unit esu/cm² for surface charge density can be changed into meq/cm² by taking into consideration that 1 coulomb = 3×10^9 esu, and 1 faraday = 96,500 coulombs/g eq.

However, not much information is available yet on the application of the surface charge density equation in humic matter.

9.3 Electric Double Layer

The concept of double layers is always discussed in relation to charged clay surfaces, and no information is available that it also pertains to charged surfaces of humic matter. The present author cannot find any reason why double layers cannot also exist at the surfaces of humic substances. Both clays and humic matter are colloids that are negatively charged. As stated in an earlier section, the negative charges of humic substances are even substantially higher than those of clays. Hence, the surfaces of humic substances will also attract *counterions* in the same way as the clays. These counterions are attracted similarly by negative charges, and it makes no difference whether the negative charges come from the clay or humic matter surfaces. The issue lies perhaps more in the fact that not much research has been conducted on double layers in humic acids, which is also the case with surface charge densities as stated above.

Because of the presence of electronegative charges, the colloid surface, in general, can attract cations. These positively charged counterions are held at or near the colloid surface; hence, the negatively charged surface is screened or covered by an equivalent cloud or swarm of counterions. This is nature's way of maintaining electroneutrality in the soil's ecosystem. Together the negatively charged surface and the swarm of counterions in the liquid phase are called the *electric double layer*. Theoretically the negative charge is a localized point charge within the solid surface, as indicated earlier, but customarily this charge is considered to be distributed uniformly over the colloidal surface. The distribution zone of the counterions in the liquid phase varies according to the theories existing on electric double layers. At the state of present knowledge, four theories are available in the literature:

(1) Helmholtz; (2) diffuse double-layer theory of Gouy and Chapman; (3) Stern double-layer theory; and (4) triple-layer theory of Yates, Levine, and Healy. Since these theories are well covered in the literature, for those interested reference is made to Tan (1998, 2011) and other basic soil chemistry textbooks.

9.3.1 Fused Double Layer

In the existing theories on electric double layers, the concepts presuppose that two particles in suspension approaching each other will repel each other because the outer zones of their double layers are equally positive in charges. Such a repulsion prevents the colloidal suspension from flocculating, and the suspension is called stable. Flocculation by interparticle attraction can only occur when the double layers are suppressed to very thin layers by, for example, increasing the concentration of counterions. The thin double layers then decrease the interparticle distance between the approaching particles, making a close approach possible. If the interparticle distance decreases to $\leq 20 \text{ \AA}$, the theories assume that the van der Waals attraction becomes larger than the repulsive forces, and this results in flocculation of the particles.

The present author is of the opinion that the presence of electric double layers surrounding individual colloidal particles is only possible in very dilute condition or very thin soil suspensions, containing only very small amounts of particles. This condition allows the particles to remain in suspension as true individual particles, each exhibiting electric double layers. The thick double layers separate them from each other by considerable distances. In natural conditions, even minor puddling of soils causes dispersion of relatively large amounts of organic and inorganic colloids. These particles, each surrounded by their counterion clouds, are close to each other. However, the double layers are, in fact, not repelling the particles, but two double layers, confronting each other, are more likely to fuse together to become just one layer. This *fused double layer* is shared by the two adjacent particles in question. The negative surface of one particle is unable to distinguish whether the counterions belong to its own or to the neighbor's surface. Neither can the counterions. Squeezed between two adjacent surfaces, they are unable to distinguish to which charged surface they actually belong. This conforms to the concept of cation exchange, which dictates that, for example, Na^+ ions from one surface can freely exchange for Na^+ ions from the other surface. For more details and further implications of the fused double-layer concept, the interested reader is referred to Tan (2011).

9.4 Chemical Reactions and Interactions

Because of the presence of electrical charges and electrochemical properties, as discussed in the preceding sections, a number of reactions and interactions can take place. At low soil pH, the humic molecule is expected to exhibit positive charges of importance in phosphate fixation and other types of interactions with anionic substances. At a pH range common in most natural and agricultural soils, the humic substances are more likely negatively charged and are capable of adsorption or attracting cations, which leads to cation exchange reactions. When both the carboxyl and phenolic-OH groups are completely ionized or dissociated, humic matter is able to undergo complex and chelation reactions with metal ions or other soil constituents, both xenobiotics and natural compounds (Figure 9.2). These reactions play an important role in soil fertility, plant nutrition, and detoxification of soils and in enhancing environmental quality, as will be discussed in more detail in Chapter 11. Both adsorption and complex reactions can also take place by a water and metal bridging reaction. This is the process by which two negatively charged soil constituents can attract each other. The interaction between humic acid and clay, made possible by water or metal bridging, is also called *coadsorption*. It is reported to also play an important role in adsorption of phosphate ions. Water or any of the metal ions, Ca^{2+} , Al^{3+} , Fe^{3+} , Fe^{2+} , and Mn^{2+} , can serve as a bridge between the organic ligand (humic substance) and the clay micelle. Sodium, Na^+ , formed by fusing of two opposing electric double layers, was explained earlier (Tan, 2000) to play an important role in interparticle attraction and repulsion.

Each of these reactions will be discussed in more detail in the following sections.

9.4.1 Adsorption

The electrochemical properties discussed in the preceding sections find many practical applications in soils. Besides the beneficial effect of flocculation on soil conditions and plant growth, they are why soils develop the capacity to adsorb gas, liquid, and solid constituents. Cation exchange reactions, interactions between clay and organic compounds, including complex reactions and chelation between metal ions and inorganic and organic colloids, are additional implications of the electrochemical behavior of soil colloids. The latter reactions are more pronounced in humic substances than in clay minerals. Not only are adsorption and cation exchange exhibited

much more by humic substances, but also complex and chelation reactions are within the active chemical domain of humic matter.

In contrast to the above, the rate of a true chemical reaction increases as temperature is increased, as formulated by the law of van't Hoff. Therefore, these differences can be used to distinguish an adsorption process from a true chemical reaction, although a similar equilibrium can be reached in the latter.

9.4.1.1 Specific and Nonspecific Adsorption

Recently, the tendency exists to refer to *specific adsorption* for complexation of solutes by inner-sphere surfaces of clay minerals and *nonspecific adsorption* for complexation of solutes by outer-sphere surfaces of clays (Sposito, 1989; Zachara and Westall, 1998). If a solute or ion does not form a complex with the charged surface of clay, it is believed to be adsorbed in the *diffuse-ion swarm*. This issue will be addressed in more detail in the next section.

9.4.1.2 Positive and Negative Adsorption

A great deal is known about the concept of adsorption by inorganic soil constituents or clay minerals, but not much research data are available on adsorption by humic acids and the like. The theory of adsorption in soils is more concerned with the type of concentrating material at the solid–liquid interfaces of clay minerals, as manifested by the counterions in double-layer positions. This type of adsorption is often distinguished into *positive* and *negative adsorption*. Positive adsorption is defined as the concentration of solutes on the clay mineral surfaces. It is also referred to as specific adsorption. The solute usually decreases the surface tension. On the other hand, negative adsorption is the concentration of the solvent on the clay surface, and the solute is then concentrated in the bulk solution. In this case, surface tension is increased. Since clay minerals are usually negatively charged in ordinary soil conditions, cationic counterions are subject to positive adsorption, whereas anions will be mostly affected by negative adsorption. For more details on the subject, reference is made to Tan (1998) and Gortner (1949).

Since humic substances obey similar rules in the development of electrical charges as the clay minerals, it is perhaps fair to expect that they will also exhibit the same two types of adsorption processes as do the clay minerals. The following information provides additional support in this aspect. The soil pH and pK_a of organic adsorbates have been reported to affect the

extent of negative and positive adsorption of these organics by negatively charged clay minerals (Frissel, 1961; Bailey and White, 1970). In general, it is noted that negative adsorption is dominant at soil pH >4.0, whereas the organics are positively sorbed at soil pH ≤4.0. Therefore, negative adsorption of organic substances seems to occur first until the pH in the soil approaches the pK_a value of the adsorbates, after which (or below which) positive adsorption takes place and increases as the soil pH decreases. According to Bailey and White (1970), positive adsorption starts when the soil pH is approximately 1.0 to 1.5 pH units higher than the dissociation constant of the organic compounds.

In view of the discussions above, it is perhaps clear that a soil pH of 4.0 is above the pK_a of humic matter; hence, the humic substances are by rule mostly negatively charged, causing, in their role as the adsorbates, their repulsion by the also negatively charged clay surfaces. The use of a limit of soil pH 3.0 is perhaps better, instead of 4.0, since this corresponds to the start of dissociation of COOH groups as explained before. Therefore, in now considering humic matter as the adsorbent, its negative charge attracts cations by positive adsorption as expected at soil pH >3.0, and at the same time causes negative adsorption or repulsion of anions. However, the negative charge of humic acids will decrease with a decrease of soil pH, and the charge will become positive if the soil pH decreases to <3.0, or the dissociation constant, pK_a, of the COOH group in humic matter. This is then the condition where positive adsorption of anions can become significant.

9.4.1.3 Adsorption Characteristics

Adsorption reactions are defined as reversible and equilibrium reactions (Gortner, 1949). Sometimes an adsorption process results in chemical changes of the adsorbed material. The changes are of such a nature that desorption is inhibited; hence, the process is neither reversible nor in equilibrium. This type of adsorption is called *pseudo-adsorption*.

Another important characteristic is that adsorption generally decreases as temperature increases; in other words, adsorption is less at elevated temperatures. This is caused by increased kinetic energies of the molecules at higher temperatures, interfering with the concentrating process. To illustrate this issue, the results of adsorption of fulvic acids by a Cecil soil, a Typic Hapludult, in Georgia, United States, are provided in Figure 9.3 (Tan et al., 1975). The isotherms for adsorption at 25°C, 35°C, and 50°C show adsorption of fulvic acids by the Cecil soil to decrease with increased temperature.

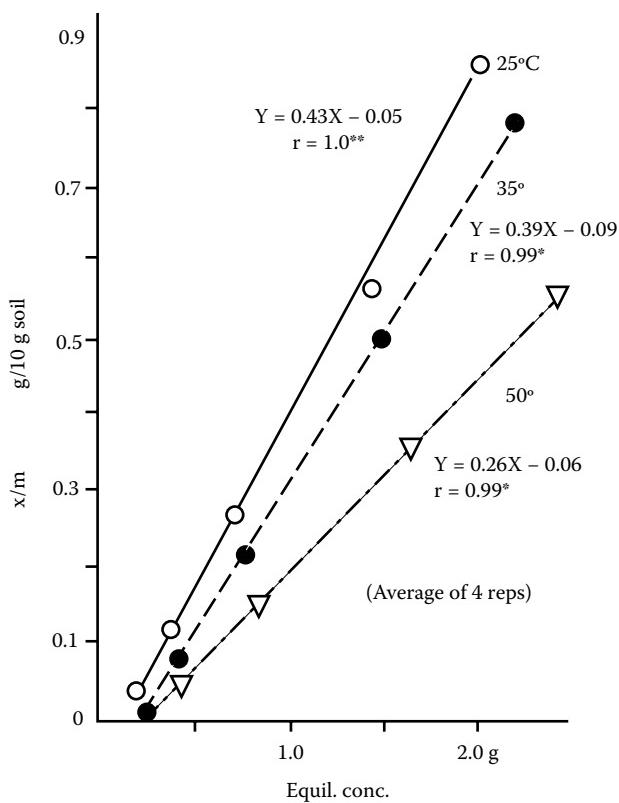


Figure 9.3 Adsorption of fulvic acid, extracted from broiler litter, by a Cecil topsoil at 25°C, 35°C, and 50°C. ○ = 25°C; ● = 35°C; ▽ = 50°C. (From Tan, K. H. et al., *Environ. Sci. Technol.*, 9, 132, 1975.)

In contrast to the above, as mentioned earlier, the rate of a true chemical reaction increases as temperature is increased, as formulated by the law of van't Hoff. Therefore, these differences can be used to distinguish an adsorption process from a true chemical reaction, although a similar equilibrium can be reached in the latter.

As indicated earlier, the tendency exists to refer specific adsorption to complexation of solutes by inner-sphere surfaces of clay minerals and non-specific adsorption for complexation of solutes by outer-sphere surfaces of clays (Sposito, 1989; Zachara and Westall, 1998). If a solute or ion does not form a complex with the charged surface of clay, it is believed to be adsorbed in the diffuse-ion swarm. The formidable statistics accompanying these new developments have convinced many scientists to jump eagerly onto the bandwagon. However, to a large number of other scientists, they only result in making the subject more complex and confusing. Questions

are often raised about the inner- and outer-space surfaces in clay minerals and especially in organic compounds (Tan, 2003b). Many also wonder what the difference is between a diffuse-ion swarm and ions “complexed” by outer-sphere surfaces. Complexation of ions by outer-sphere surfaces is defined as nonspecific adsorption attributed to electrostatic attraction. However, this is also the definition of diffuse-ion swarm. Complexation of ions by inner-sphere surfaces makes the confusion worse, because in the triple-layer theory adsorption in inner spheres is limited to adsorption of potential determining ions only, creating the so-called effective surface. The charge is usually reversed, since the effective surface carries the charge of the adsorbed potential determining ions. All the unanswered questions above find their origin perhaps in regarding adsorption as similar to complex reactions. In basic chemistry, complex reactions are usually considered to occur only with certain cations, and in particular with the transition metals, Al, Fe, Mn, Cu, and Zn, binding organic compounds. These reactions, yielding the so-called metal-organo complexes, are to be viewed as rather different from the adsorption of cations in a double-layer region of clay surfaces. The complexed ion usually assumes a central position, and the coordination number of the metal determines the number of organic molecules complexed (Murmann, 1964; Mellor, 1964). Unless another definition is available, the concept of complexation in basic soil chemistry differs from that of adsorption in inner- and outer-sphere surfaces as discussed above.

9.4.1.4 Adsorption Models

Several models are available for describing adsorption processes in soils; some are simple and others are complex. Although most of them have been developed for inorganic compounds, in view of the presence of similar electrochemical properties, there is no reason why the models cannot also apply to organic compounds, such as humic substances. Since adsorption is an equilibrium reaction, fundamental principles of soil chemistry, such as the law of mass action or the law of equilibrium, have been applied for interpretation of the process, which is considered as the scientific approach. Apparently this method has yielded mixed results because of the extreme difficulties obtained when attempts were made to extend it by involving the double-layer concept. In contrast, another group of methods tries to explain adsorption by just accepting the facts obtained without relating them to any basic chemical principle. This second group is called the empirical method, which includes the Freundlich and Langmuir equation models. Since the

latter two are well-established models and closely related to one another, only the Langmuir model will be provided below as an example:

$$\frac{x}{m} = \frac{k_1 C}{1 + k_2 C} \quad (9.25)$$

where x = amount adsorbed, m = amount of adsorbents, k_1 and k_2 = constants, and C = concentration in equilibrium solution.

At low concentrations, the value of $k_2 C$ becomes so low compared with the factor 1 that it can be neglected, and Equation 9.25 reverts to the Freundlich model: $x/m = k_1 C^{1/n}$, in which $1/n = 1$. The Freundlich equation suggests adsorption of solutes to increase indefinitely, whereas the Langmuir indicates that at high values of C , adsorption reaches a maximum. The latter corresponds more to soil conditions where the capacities for adsorption and ion exchange are noted to become saturated.

Another method of describing adsorption processes is by the identification of shape and curvature of adsorption isotherms. In this respect, four basic types of adsorption models have been recognized, e.g., S-, L-, C-, and H-type isotherms (Weber, 1970; Giles et al., 1960). The S- and L-type adsorption curves are considered to predict similar processes as the Langmuir isotherm (Choudry, 1983). A detailed discussion on these adsorption isotherms and other classical adsorption models, e.g., Brunauer, Emmett, and Teller (BET) and Gibbs, is provided by Tan (1998).

Recently, several scientists have regarded adsorption as identical to cation exchange reactions. Impressive names have been used to redistinguish adsorption, e.g., surface complexation nonelectrostatic model (SC-NEM), surface complexation electric double-layer model (SC-EDL), mechanistic, and semiempirical approach (Zachara and Westall, 1998). In this new approach, adsorption in inner- and outer-sphere surfaces is redefined as a complex reaction, forming a stable molecular unit when an aqueous species reacts with a surface functional group. For more details on the merits of these redefined concepts on adsorption, reference is made to Tan (2003b), since modeling of adsorption processes is more the subject of soil chemistry than the science of humic matter.

9.4.1.5 Forces and Mechanisms of Adsorption

Forces responsible for adsorption reactions include (1) physical forces, (2) chemical forces, (3) hydrogen bonding, (4) hydrophobic bonding,

(5) electrostatic bonding, (6) coordination reactions, and (7) ligand exchange. Such a listing, compiled from several textbooks and journal articles, is subject to many arguments. Since the issue has been examined in detail earlier (Tan, 1998, 2003a), this section will discuss the material related to the forces and adsorption mechanisms exerted by humic substances only. In this respect, the role of humic matter as an adsorbent should be clearly distinguished from that as an adsorbate.

As an adsorbent, humic substances attract solutes or cations in a similar fashion as negatively charged clay minerals. As an adsorbate, the humic compounds are then attracted by the clay minerals. Not much research has been conducted on these adsorption reactions by humic acids, and much of the discussions below are based on conjecture or scientific reasoning.

9.4.1.5.1 Physical Forces

A major physical force is the van der Waals force, which is active at close distance among all types of molecules. It is additive in nature and hence will increase in force with an increase in size of the compounds or an increase in molecular weight, such as humic acid. Van der Waals forces decrease rapidly with interparticle distance. They are also believed to be especially important in nonpolar organic compounds and neutral organic substances.

9.4.1.5.2 Electrostatic Bonding

This type of reaction occurs between two molecules that are opposite in charges. The negatively charged humic molecule will attract cations, and these ions create the so-called counterion atmosphere of the double layer. Since these counterions can be easily exchanged by other similarly charged ions, they are considered also exchangeable cations. Therefore, an adsorption process may develop into the process of an exchange reaction, when an exchange takes place. The new theories consider adsorption identical to exchange reactions, as pointed out earlier. However, it must be realized that adsorption is not the same as exchange reaction, since the counterions in the double layer can remain in the adsorbed condition. This type of reaction is indeed closely related to exchange reactions, but then it is also related to adsorption by H-bonding and ligand exchange.

Electrostatic bonding is the reason for (1) adsorption of water, (2) adsorption of cations leading to cation exchange reactions, and (3) adsorption of organic compounds. The latter may not only develop into complex reactions, but also into a reaction involving an exchange of the adsorbed organic ligand for an inorganic cation, called *ligand exchange*.

9.4.1.5.3 Chemical Forces

The following discussion is limited only to protonation since many other forces can also be considered chemical forces. Protonation can occur at the humic molecule surface, and in the solution phase, as well as in the hydration shell of cations. It is of importance for adsorption of anions and organic compounds that are basic in nature. Ammonia, NH_3 , is noted to be chemisorbed by clays in the form of NH_4^+ , which is considered a protonated form of NH_3 (Mortland et al., 1963). The protons are provided by the dissociation of adsorbed water or by water in the hydration shell of cations. Protonation of basic organic compounds has been noted to occur by clay minerals saturated with H and/or Al. For a more in-depth discussion, reference is made to Tan (1998).

9.4.1.5.4 Hydrogen Bonding

The bond by which a hydrogen atom acts as the connecting linkage is called a hydrogen bond. As such it is perhaps clear that hydrogen bonding is very closely related to protonation. However, protonation involves a full charge transfer from the electron donor (base) to the electron acceptor (acid), whereas hydrogen bonding is a partial charge transfer only (Hadzi et al., 1968). Hydrogen bonding is an important adsorption force for humic substances because of the existence of functional groups containing hydrogen in their molecules, e.g., N-H, $-\text{NH}_2$, $-\text{OH}$, and COOH groups. Hydrogen bonding then takes place between these functional groups and the oxygen on clay mineral surfaces.

9.4.1.5.5 Hydrophobic Bonding

This is the type of bonding associated with adsorption of nonpolar molecules, where the latter compete with water molecules adsorbed on the adsorption sites. In the process, the adsorbed water is expelled by or exchanged for the nonpolar molecule, which is the reason for calling the process hydrophobic. Polysaccharides, for example, are adsorbed in this way by clay minerals, and the expulsion of water, especially from the intermicellar clay surfaces, reduces swelling.

9.4.1.5.6 Coordination Reaction and Complex Formation

The reaction involves coordinate covalent bonding, in which the ligand donates electron pairs to the metal ion. The latter is usually a metal in the series of *transition metals*. The ligand, therefore, fits the definition of a *Lewis base* and the metal is then the *Lewis acid*. The compound formed is called a coordination compound, complex compound, or an organo-metal complex.

Coordination compounds or organo-metal complexes are substances containing the metal as a central atom, surrounded by a cluster of organic ligands. The total number of ligands that can be complexed corresponds to the coordination number of the metal.

The terms inner-sphere and outer-sphere complex reactions have been used recently to indicate adsorption processes by these types of complex reactions. As discussed previously, inner-sphere complex reactions are referred to as adsorption processes. It is perhaps a special type of adsorption that does not obey the rules of reversibility and desorption. The desorption process is often only partially possible, and is also reported to occur only with other transitional metals at a very specific pH value (Lindsay, 1974; Lindsay and Norvell, 1969). For example, Na^+ cannot be exchanged for Fe^{3+} and cannot assume the central position in the complex compound. It is up to the reader whether or not to accept the inner-sphere theory as an adsorption process.

9.4.1.5.7 Ligand Exchange

This process entails the replacement of a ligand by an adsorbate molecule. The adsorbate can be an inorganic ion or an organic molecule; however, in either case, it must have a stronger chelation capacity than the ligand to be replaced.

9.4.2 Cation Exchange Capacity

The CEC of soils is defined as the capacity of soils to adsorb and exchange cations. The basic concepts on CEC have been provided elsewhere in detail (Tan, 1998). Scientifically, CEC is related to the surface area and surface charge of clay minerals or humic substances. This relationship is expressed by the following equation, which serves as a statistical model for CEC:

$$\text{CEC} = S \times \sigma \quad (9.26)$$

in which S = specific surface (cm^2/g) and σ = surface charge density (meq/cm^2).

The surface charge density can be calculated by using Equations 9.23 and 9.24. It is usually expressed in esu/cm^2 ; however, as explained before, this unit can be converted into meq/cm^2 . However, it is common practice in the determination of CEC to analyze all exchangeable cations, and the CEC is then

$$\text{CEC} = \Sigma \text{ meq exchangeable cations per } 100 \text{ g soil} \quad (9.27)$$

The magnitude of the CEC of humic substances is considered related to their total acidity value. As discussed in Chapter 5 and other sections of this book, the average values for total acidity vary from 6.0 to 8.9 meq/g for soil humic acids, and from 10 to 12.3 meq/g for fulvic acids.

In the past, two types of CEC for humic substances have been distinguished: (1) a *measured CEC*, determined by exchange with any suitable cation, and (2) a *potential CEC*, which is defined as the sum of the measured CEC and the CEC attributed to blocked sites (Schnitzer, 1965b). These blocked sites will be exposed by liming or when the humic substances are extracted from the soil, and their CEC is often larger than that of the measured CEC (Piccolo and Stevenson, 1981). However, a more recent concept recognizes a total CEC, defined as

$$\text{CEC}_t = \text{CEC}_v + \text{CEC}_p \quad (9.28)$$

in which the subscripts t = total, v = variable, and p = permanent (Tan, 1993, 2011).

Humic substances are believed to contain a very small amount of permanent charges, responsible for the development of CEC_p . These charges are estimated to amount to only 10% of the total negative charges, whereas the remaining 90% are attributed to the variable charges (Brady, 1990; Tan, 1993). The CEC_t is formerly called CEC_m , in which m = maximum (Mehlich, 1960). In the case of clay minerals, the latter is measured at pH 8.2, and no further increases in CEC values can be obtained by analysis at higher pH values. If this is true, then it can be expected that the variable negative charge reaches a maximum value at pH 8.2. This is in disagreement with the formation of negative charges based on the theory of dissociation of functional groups in humic matter, as postulated by Posner (1964). As discussed earlier, Posner believes that the carboxyl groups dissociate their protons at pH 3.0, whereas the phenolic-OH groups are ionized at pH 9.0. Consequently, negative charges in humic matter are more likely to increase at pH values >8.2, which is in contrast to clay minerals.

Several scientists, in fact, believe that contrary to clay minerals, humic substances do not possess limits in their CECs. Stevenson (1994) is also of the opinion that the exchange capacity of humic matter increases markedly with pH, which is attributed to increased ionization of COOH groups at high pH values. The latter differs sharply from Posner's findings above, and also deviates from basic rules in chemistry where COOH groups are listed as strong acidic groups with low pK_a values. Formic acid, a carboxylic acid with the

formula of HCOOH, has a pK_a of 3.62, and peruvic acid with a formula of CH_3COCOOH exhibits a pK_a of 2.50 (Conn and Stumpf, 1967), all testifying to a strong acidic behavior. It is the phenolic-OH group that is responsible for the increase in CEC of humic substances at high pH. This group is known to behave as a weak acid, and its half-dissociation point is apparently at $pK_a = 9.0$, whereas 99% dissociation was expected to be attained at pH 11.0, as discussed before. Consequently, the CEC of humic substances will correspondingly increase with the increase in negative charges due to increased dissociation of phenolic-OH groups at high pH. However, the present author cautions against using extremely high pH values, 12.0–13.0, because of the danger for humic substances to be broken down by hydrolysis.

9.4.2.1 Cation Exchange Mass Action Law Models

In soil chemistry, a number of cation exchange reaction models have been developed mostly for application with clay minerals. Similarly as with adsorption models, there is no reason why the exchange reaction models cannot be applied to exchange reactions of humic substances. However, since modeling is more in the realm of soil chemistry rather than in the science of humic substances, the most common models will only be discussed as examples in these sections, e.g., the models based on the law of mass action. The purpose is to show that the same statistical modeling process can be applied for exchange reactions with humic acid as well as with clay minerals. It is also meant to underscore that different models are often created and used for similar exchange reactions occurring with similar principles, but only written differently and expressed by a different arithmetic or computational formula only. After all, according to Yale Hirsch, “a statistician is a scientist who can draw a straight line from an unwarranted assumption to a foregone conclusion.” For details on the other models, such as the empirical equations, kinetic equations, the Donnan equation, and thermodynamic equations of cation exchange, reference is made to Tan (1998, 2011).

Two of the most common models in the group based on the *law of mass action* are the Kerr and Gapon equations that find extensive application in cation exchange chemistry. The two models are, in fact, closely related to each other as can be noticed from the following discussion. Let us assume that a mono-divalent cation exchange reaction at equilibrium can be written as follows:



in which HA = humic acid. According to the law of mass action, the equilibrium constant, K_{eq} , of the reaction is then

$$K_{eq} = K_{ex} = \frac{[Na^+]^2(Ca^{2+})}{(Na^+)^2[Ca^{2+}]} \quad (9.30)$$

The signs [] and () denote adsorbed and free cations, respectively. Because this is an exchange reaction, K_{eq} can also be considered the exchange constant, K_{ex} . Equation 9.30 is called the *Kerr equation*. By taking the square root, Equation 9.30 changes into

$$\sqrt{K_{ex}} = K'_{ex} = \frac{(\sqrt{Ca^{2+}})[Na^+]}{\left[\sqrt{Ca^{2+}}\right](Na^+)} \quad (9.31)$$

Equation 9.31 is known as the *Gapon equation*.

9.5 Complex Reaction and Chelation

These reactions are the outstanding properties of humic substances that have made them very conspicuous in soils, agricultural, pollution, and environmental issues. It is these reactions that have propelled humic substances to be regarded as one of the most active components in soils, not matched by other soil constituents.

The terms complex formation and chelation have often been used in soil science interchangeably. In the preceding sections above, it has been pointed out that complex formation has also been considered by some to be identical to the adsorption processes in inner- and outer-sphere regions of clay minerals. Consequently, it is deemed necessary to provide the common definitions for these processes as they appear in many textbooks. Owing to the nature of reaction and bonding processes, a distinction must be made between adsorption, complex formation, and chelation. The difference from adsorption has been discussed earlier, and at present the focus will be on concepts and definitions differentiating complex formation from chelation.

9.5.1 Complex Formation and Coordination Compounds

Complex formation or complexation is the reaction of a metal ion with a compound through electron-pair sharing (Murmann, 1964; Mellor, 1964; Martell and Calvin, 1952). The resulting product is called a *metal coordination compound*, which can be in the form of cations, anions, or neutral molecules. The metal is the electron-pair acceptor, whereas the compound is the electron-pair donor. The latter is usually called a ligand, and may assume the form of an anion (HA^-) or a neutral molecule (NH_3). The metal ion serves as the central atom, and the ligands are coordinated around it in a first coordination sphere. The number of ligands bonded to the central atom in a definite geometry depends on the coordination number of the metal. Almost any metal atom can serve as an acceptor atom, including K^+ , Na^+ , Li^+ , Ag^+ , and Au^+ . A long-known complex compound with a monovalent ion is potassium ferrocyanide. However, the most common metals capable of complexation reactions are the transition metals, e.g., Al, Fe, Cu, Mn, and Zn.

Mention is also made in the literature of distinguishing two categories of metals on the basis of differences in forming coordinate covalent bonding (Stevenson, 1994). Category A includes metals capable of forming complexes with ligands containing oxygen as the electron-pair donor. Category B is a group of metals that preferentially coordinate with ligands containing N, P, and S. Not much is known about this preferential nature of metals for ligands, and more information is required to accept or reject the contention above.

9.5.2 Chelation

Chelation is the reaction when a ligand can bind the metal ion with two or more donor functional groups to form a ring structure. The compound formed with the characteristic heterocyclic ring is called a chelate (Greek *chele* = lobster claw), which, in fact, is referring to the pincer-like bonding of the metal (Figure 8.2, middle). If one ligand is involved in the formation of a chelate, the compound is called a monodentate chelate, and when two ligands form a chelate, the compound is called a bidentate, etc. For more details, reference is made to the references given above and to Tan (1998).

The reactions in Figure 9.4 are provided to illustrate and underscore the differences between adsorption, complexation, and chelation between a metal (Zn) ion and humic acid. The complexation reaction appears to be similar to an adsorption reaction on paper. However, adsorption is an

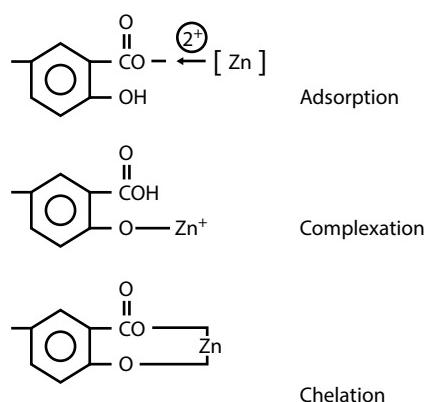


Figure 9.4 Adsorption, complex formation, and chelation of Zn by humic acid.

electrostatic attraction of Zn^{2+} by the negative charge of the humic acid surface, whereas complexation is a coordinate covalent bonding through electron-pair sharing. In this case, the Zn ion is bonded and becomes an integral part of the humic acid molecule, as indicated on Figure 9.4. The chelate as illustrated is an example of a monodentate.

9.5.3 Significance of COOH Groups in Complexation

Recently, the idea has surfaced that formation of metal complexes is attributed to the interaction of the metal with the COOH groups. From a study on photochemical and microbial processes, affecting metal–humic interactions, McKnight et al. (2001) have arrived at the conclusion that the carboxyl groups account for the majority of strong complexing sites in the humic molecules. These functional groups can form charge transfer complexes with metals such as Fe and Cu.

The COOH groups can be linked to the aromatic or to aliphatic C structure. Humic substances, which are highly aromatic, may contain large amounts of aromatic-COOH groups; hence, according to the authors above, they will exhibit strong complexing sites for Cu. The COOH groups on the aliphatic sites are allegedly important for complexing especially Ca ions. Fulvic acids isolated from rivers with “soft water” (low Ca and Mg contents) are believed to have stronger Cu-binding sites than fulvic acids extracted from rivers containing “hard water” (Breault et al., 1996; Leenheer et al., 1998).

9.5.3.1 Complexing Constant and Capacity (CC)

Simple sorption, or simultaneous coprecipitation, and adsorption of humic substances on the Fe oxide surfaces have also been considered complex reactions by McKnight et al. (2001). Variations in the Fe complexation constant among carboxylic groups are believed to influence these reactions. To identify Fe complexation reactions as similar to precipitation or coprecipitation and adsorption is confusing. First, a definition must be provided by the authors for their Fe complexation constant. In the literature, a stability constant for chelates is available but no information can be found about an Fe complexation constant. Perhaps, the authors meant the *affinity* of the various carboxyl groups for Fe, which has a totally different meaning. Purdue (1988, 2001) has mentioned *complexing capacities (CC)* of humic acids, which are related to the total acidities (TA) of the humic compounds, and proposes to define it as follows:

$$CC = TA/z \quad (9.32)$$

where z = valence of the cation. His reasoning in including the valence of the cation is that the electrostatic driving force for complexation of a cation by humic substances is gradually decreased as the negative charge of the metal-humic complex decreases to zero. The latter is usually reached when the humic compound becomes fully saturated with metal ions. Although it is a very sound concept, not much information is available and more research is required to test the suitability of using CC in complex reactions by humic substances.

9.5.3.2 Issue of Adsorption, Precipitation, and Complex Reaction

Second, precipitation and “simple” adsorption have been interpreted by the authors as coordination reactions. As far as the rules are concerned in basic soil chemistry, precipitation takes place only with noncharged clay minerals or noncharged humic substances, and no covalent bonding is involved. This process occurs normally at the ZPC or isoelectric point values. On the other hand, adsorption is defined in basic soil chemistry as the attraction of counterions on the surfaces of charged clay minerals and humic substances. In the strict sense of simple adsorption, the process does not include electron-pair sharing, a requirement for complex reactions. It is commonly known by now that complex reactions involve covalent bonding through electron-pair

sharing. This capacity is exhibited especially by the phenolic-OH groups since they behave usually as Lewis acids. The carboxyl groups, on the other hand, are acidic in nature and behave as Brønsted–Lowry or as Arrhenius acids. Consequently, they are expected to react more through electrostatic attraction rather than through electron-pair sharing. The chemistry of the phenolic-OH and COOH groups in relation to complex reactions and chelation will be discussed in more detail in the following section.

9.5.3.3 Conditions for Chelation as Affected by pH and pK_a

As discussed in the preceding sections, the two most important functional groups in humic substances responsible for complex formation and chelation are the COOH and phenolic-OH groups. They will dissociate and become active at two different pK_a s, e.g., pK_1 and pK_2 , and/or at different soil pHs. As examined earlier, the carboxyl groups usually behave as strong acidic groups and, as explained before, exhibit pK_a values around 3.0, with a chance of 99% ionization at a soil pH of 5. Bonding by this group constitutes only adsorption; however, according to McKnight et al. (2001), some complex reaction may also be exhibited.

From our studies above, it seems chelation starts to become important when the soil pH increases to a value approaching the conditions for the phenolic-OH groups to ionize. As stated earlier, at pH 9.0, the pK_a of these functional groups is reached at which ionization is expected to be 50%. Therefore—considering both dissociations of COOH and phenolic-OH groups—chelation is predicted to take place at pH values higher than 5.0, and increases gradually to reach high values at especially pH 11, when 99% ionization of the phenolic-OH is attained. Studies on the effect of pH on chelation seem to support the above. As a result of his investigations, Tan (1978) indicates that the amounts of Al chelated by humic acids increase from 0.226 meq/100 g at pH 7.0 to 0.445 meq/100 g at pH 11.5. The amounts of Fe chelated by humic acids are also reported to increase from 0.255 meq/100g at pH 7.0 to 0.443 meq/100 g at pH 11.5.

In conclusion, it can perhaps be stated that complex formation in humic substances occurs mostly at low soil pH, whereas chelation reaction is more dominant at higher pH values.

9.5.4 Stability Constants of Chelates

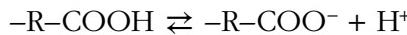
Humic matter is capable of forming soluble and insoluble complexes and chelates (Tan, 1978; Stevenson, 1976a, b). Some of the factors affecting this

solubility are the (1) type of humic substance, (2) types of metals, (3) dissociation of functional groups, and (4) saturation of binding sites.

Metal complexes of fulvic acids are, in general, more soluble than those of humic acids. This is perhaps attributed to the lower molecular weights and higher solubility of fulvic acids.

Several of the cations have the potential to enhance polymerization of humic matter by linking the individual molecules together into chainlike structures. The metal–humic acid complex remains soluble when the metal/humic ligand ratio is low (Stevenson, 1994). The metal–humic acid complex becomes insoluble and precipitates as the metal bridges increase and the chain-like structure grows. The maximum chelating or complexing capacity of humic matter equals its total acidity, which is the amount of H^+ ions from both COOH and phenolic-OH groups. A total acidity of 1000 meq/100 g corresponds to 90 mg of Al^{3+}/g of humic acid bonded by the functional groups. If this maximum capacity is satisfied, the humic ligand is saturated with the metal, and the complex or chelate becomes insoluble.

Dissociation of functional groups induces repulsion of charged groups, as indicated by the dissociation of carboxyl groups as follows:



As shown in the reaction, it gives the humic molecule a stretched configuration. After reacting with a cation, the negative charge is neutralized and the stretched molecule collapses, thereby reducing solubility.

The problem of solubility of metal–humic acid complexes and chelates is usually expressed in terms of stability constants. These constants are derived as follows. Assume that the following complex or chelation reaction occurs:



in which M^{2+} = divalent cation, HA = humic acid, $M-(2A)$ = bidentate metal chelate.

According to the law of mass action, the equilibrium constant of Equation 9.33 is then

$$K_{eq} = \frac{[M-(2A)][H^+]^2}{(M^{2+})(HA)^2} \quad (9.34)$$

By taking the log, this equation changes into

$$\log K_{\text{eq}} = \log \frac{[M - (2A)][H^+]^2}{(M^{2+})(HA)^2} \quad (9.35)$$

If the activities of HA and M-(2A) chelate are assumed unities at standard state, then

$$\log K_{\text{eq}} = \log \frac{(H^+)^2}{(M^{2+})} \quad (9.36)$$

or

$$\log K_{\text{eq}} = 2 \log (H^+) - \log (M^{2+}) \quad (9.37)$$

where $\log K_{\text{eq}}$ is called the stability constant. It determines the solubility and stability of the metal chelates formed (Tan et al., 1971a, b). The solubility of chelates is low, or, in other words, the stability of chelates is high, if the value of $\log K_{\text{eq}}$ is high. These authors have calculated $\log K_{\text{eq}}$ values for metal–fulvic acid chelates, and some of the data are shown in Table 9.2. These $\log K_{\text{eq}}$ values are in the range of those reported by Stevenson (1994) for his 1:1 complexes of Cu and Zn with fulvic and humic acids. The data in the table indicate that comparatively the value of $\log K_{\text{eq}}$ decreases from Mg–FA → Zn–FA and to Cu–FA chelates, suggesting that the Cu–FA chelates are more stable (or less soluble) than Zn–FA and/or Mg–FA chelates.

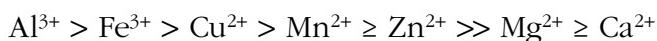
The effect of pH is also clearly demonstrated and supports the opinion stated earlier that chelation will increase at higher pH levels.

The concept of stability constants for humic chelates discussed above is used in relation to the principle in equilibrium reactions that the equilibrium

Table 9.2 Stability Constants ($\log K_{\text{eq}}$) of Metal–Fulvic Acid Chelates

| <i>Stability Constant</i> | <i>Cu–FA</i> | <i>Zn–FA</i> | <i>Mg–FA</i> |
|-------------------------------|--------------|--------------|--------------|
| $\log K_{\text{eq}}$ (pH 3.5) | 7.15 | 5.40 | 3.42 |
| $\log K_{\text{eq}}$ (pH 5.5) | 8.26 | 5.73 | 4.06 |

constant represents, in general, the rate of reaction. As depicted in Equation 9.34, the value of K_{eq} increases if the amount of chelates formed increases. Since at high values of K_{eq} , reaction 9.33 is not going to the left, the assumption is made that the chelate is not dissociating into its metal and ligand components; hence, the chelate is said to be stable. However, other interpretations are presented in the literature, and the stability constant is, for example, understood by other scientists to provide an index of the affinity of the cation for the ligand (Stevenson, 1994). However, affinity is related more to the attractive force of the ligand for the metal with the connotation of a preferential or selective attraction. Although not much information is available in this respect, the present author's unpublished files indicate that, in general, the affinity of humic acid for reaction with cations is in the following decreasing order:



Given the choice, Al^{3+} will be chelated first over Ca^{2+} , although it is then also true that the Al-HA chelate exhibits a higher stability constant, K_{eq} , than Ca-HA chelate. In other words, Ca-HA chelates are more soluble than Al-HA chelates. Metal ions, such as Al and Fe, that under normal conditions are insoluble, are soluble in chelate form and participate in soil genesis since they can now be translocated and accumulated in different soil horizons in the pedon.

Complex formation and chelation have an important impact on soil formation, soil fertility, and other issues in soils and the environment. Their role in soil formation will be discussed in the next section, whereas their importance in soil fertility will be addressed in more detail in Chapter 10.

9.5.5 Effect on Soil Genesis

Complex reaction and chelation of humic substances may affect a variety of processes related to soil formation. They can accelerate the decomposition of primary minerals and affect the formation of clay minerals, soil horizon differentiation, and soil fertility. Their role in the development of soil horizons and in soil fertility is closely related to mobilization-immobilization issues of metal chelates, and will be discussed in more detail in Chapter 10. The following discussion will address the effect of chelation on the dissolution of primary minerals and the formation of clay minerals.

9.5.5.1 Decomposition of Soil Minerals

The decomposition of soil minerals by humic substances has attracted considerable attention since the early history of soil science. Long before Dokuchaiev formulated his pedological concept, soil organic acids, including humic acids, were expected to play an important role in the decomposition of rocks and minerals (Sprengel, 1826). Since then, conflicting arguments have been presented about the effectiveness of these acids in rock and mineral weathering. Mainly owing to a lack of supporting experimental evidence, a large number of scientists have questioned in the past the role of humic matter as a weathering agent (Clarke, 1911; Loughnan, 1969). However, an equally large number of authors can also be found in the literature defending the role of humic substances as a dissolution agent (Graham, 1941; Van der Marel, 1949).

With the increased knowledge of humic acid chemistry, evidence has accumulated now suggesting humic compounds to play a significant role in mineral dissolution. Today's data indicate that the acidity and chelating capacity of the humic substances can bring about the degradation of rocks and minerals (Singer and Navrot, 1976; Schalscha et al., 1967; Baker, 1973). A massive dissolution of Si, Al, Mg, Fe, and K from mica minerals by fulvic acids has been reported by Schnitzer and Kodama (1976). The extremely low pH of 2.5 employed in their analysis is responsible for the rapid dissolution, and was later confirmed by Tan (1980) from his dissolution experiments using microcline, biotite, and muscovite. The latter author notices that fulvic acid is capable of extracting 10 times more silica and 6 times more aluminum from microcline at pH 2.5 than at pH 7.0. This leads him to conclude that both the acidic effect and complex or chelation reactions have been responsible for the dissolution process (Tan, 1986). The acidic effect is especially important at pH 2.5, whereas at higher pH chelation and complex reactions seem to be more effective. The dissolution of silica, aluminum, and potassium at pH 7.0 is relatively small in the beginning but increases rapidly with time, to reach a maximum at approximately 30 days.

The data above then indicate that by forming metal-humo complexes and/or chelates, humic substances are playing an important role in the weathering process by accelerating the decomposition of rocks and primary minerals.

9.5.5.2 Formation of Clay Minerals

The dissolution products from the mineral-weathering processes by humic substances are believed to promote or inhibit formation of clay minerals.

Schwertmann and Taylor (1977) are of the opinion that crystallization of iron oxides can be inhibited by the presence of ferric–fulvic acid complexes. However, the transformation of hematite to goethite is noticed by Schwertmann (1971) to be mediated by iron–fulvic acid complexes. Direct evidence for the inhibitory effect of fulvic acid on the crystallization of iron and aluminum hydroxides has been presented by Schnitzer and Kodama (1977) and Kodama and Schnitzer (1977, 1980). The concentration of fulvic acid and the pH are noticed by these authors to have affected the crystallization process of the sesquioxide minerals. Fulvic acid, present at concentrations of 5.0 g/L, is believed to completely inhibit the formation of goethite and hematite, whereas a concentration of 0.5 g/L of FA seems to favor the crystallization of the two minerals above. According to these authors, gibbsite is formed at pH 6.0 in the absence of fulvic acid; however, the addition of fulvic acid seems to delay first and then inhibit its crystallization and formation. The conclusion is presented by Kodama and Schnitzer that it is unlikely for crystallization of sesquioxide minerals to occur in the presence of substantial amounts of Fe–FA and Al–FA complexes in the soil solution.

A similar inhibitory effect of humic substances has also been reported in the formation of allophane. The process, called *anti-allophanic effect*, has recently been noticed in the formation of allophane and imogolite in andosols. This effect is presumably attributed to chelation of Al by humic acids, decreasing the free Al concentrations in the soil solution so that it becomes inadequate for the formation of allophane and imogolite (Shoji et al., 1993; Huang, 1995; Tan, 2000).

9.5.6 Statistical Modeling

As is the case with adsorption and cation exchange reactions, the opinion exists that the processes of complex reactions and/or chelation can also be studied more accurately by using statistical models. It is believed by several scientists that humic matter exhibits a variety of nonidentical binding sites, and a model would be able to describe quantitatively the relative concentrations and the strengths of these binding sites. Three models have been developed lately for this purpose: (1) a competitive Gaussian distribution model by Dobbs et al. (1989), (2) a Model V by Tipping and Hurley (1992), and (3) a NICA (nonideal competitive adsorption) model by Koopal et al. (1994), also known as the NICA–Donnan model. Although Purdue (1998) indicates that the three models are reasonably suitable for the purpose intended, the author notes that the perceived distribution of binding sites is

unfortunately related directly to the experimental conditions and to the type of model that is forced on the experimental data.

Purdue (2001) claims that the three models above assume humic matter to contain two classes of binding sites, e.g., sites for specific binding and sites for nonspecific binding of cations. Each site's class contains multiple sites whose relative amounts are distributed symmetrically around a central log K value, in which K is the equilibrium constant of the complex reaction between the metal ion and humic acid. In Purdue's (1988, 2001) opinion, the development of the models above requires a specific knowledge of a CC of humic matter. The latter has been discussed and formulated earlier as Equation 9.32. For more details on the models, reference is made to the specific works stated above.

9.6 Bridging Mechanism

This is another outstanding reaction among those that have made humic substances important in soils and in the environment. It is perhaps the most common everyday interaction between humic molecules and almost any compound, be it neutral or electrically charged. Negatively charged components will normally be repelled by other negatively charged soil constituents, such as clay minerals, making, therefore, clay-humic matter interactions impossible. The interactions between humic acid and clay are, in fact, important processes and are responsible for most of the accumulation of humic matter in soils. The reaction between the two negatively charged soil materials is made possible through this particular bridging mechanism, made possible especially by the presence of polyvalent cations and, most important, by the presence of water only.

The humic molecule and the clay mineral in suspension are ordinarily each surrounded by water molecules, forming so-called hydration shells. One of these water molecules can serve as the connecting linkage between the humic molecule and the clay mineral. The water molecule is dipolar in nature, and one of its H (hydrogen) atoms can be bonded by the negatively charged humic molecule, whereas the other H atom will then hook up to the negatively charged clay surface. The reaction is called water bridging since the water molecule acts as a bridge between the humic molecule and the clay mineral. An illustration of such a bridging mechanism is provided in Figure 9.5. This type of bridging shows a close relation with hydrogen bonding because a hydrogen atom acts as the connecting linkage. Since two

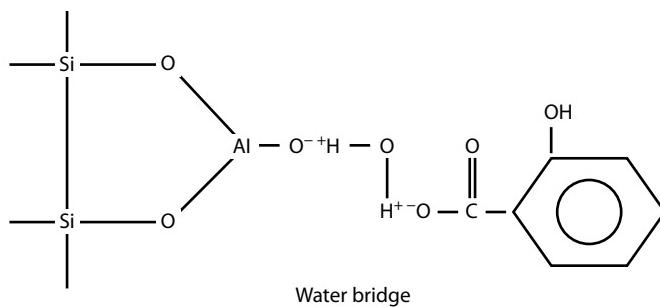


Figure 9.5 Water bridge between a negatively charged clay and humic acid.

hydrogen atoms are involved in the reaction above, it can perhaps be considered a type of complex hydrogen bonding. However, water bridging can also occur between a neutral humic molecule and a negatively charged clay surface, as shown in Figure 9.2.

Polyvalent cations are also capable of acting as bridges between the humic molecule and clay minerals. The reaction is then called *cation* or *metal bridging*. In this case, free cations in solution or metals structurally bonded in the octahedrons or tetrahedrons of clays can act as bridges. As noticed from Figure 9.6, the Al bridge (top) and the Ca bridge (bottom) are formed by the free Al and Ca ions in solution. The structural Al bridge (middle) is produced by octahedral Al on the surface of the clay mineral allophane.

Bridging is perhaps a versatile mechanism since a variety of bridging reactions are possible. As indicated above, bridging mechanisms make the reaction possible between neutral molecules, whereas several combinations between water and cations are also available.

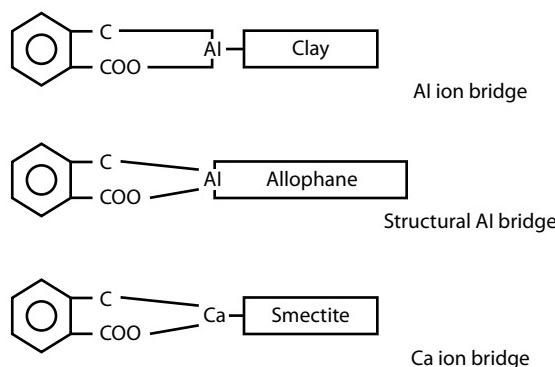


Figure 9.6 Bridging or coadsorption between humic acids and clays through metal ions in solution and structurally bonded atoms.

Anions can be bonded through bridging reactions by humic molecules, which are significant processes in detoxification reactions of xenobiotics. Several of the harmful pesticides introduced in soils are anionic in nature and can be neutralized by humic matter through binding by the process of metal bridging. Phosphate ions, which are mostly negatively charged, are also bonded either directly or through water and metal bridging by humic matter. These are topics that will be discussed in more detail in the next chapter.

Chapter 10

Agronomic Importance of Humic Matter

10.1 Importance in Soils

It is well known that soil organic matter has a favorable effect on the physical, chemical, and biological characteristics of soils. By changing the various soil properties, soil organic matter in general, and especially its humic fractions, are known to have indirect and direct effects on plant growth and crop production. Increasing soil aggregation, water-holding capacity, and improving aeration and soil permeability are some of the beneficial indirect effects in soil physics. Chemically, humic acids (HAs) increase the cation exchange capacity (CEO), providing to soils a stronger buffer capacity to resist sudden drastic chemical changes, which are important indirect effects of enhancing soil fertility and environmental quality. Toxic materials introduced into the soil by agricultural, industrial, and domestic operations will be intercepted from polluting soils and reaching the groundwater by the presence of humic acids with their huge cation exchange capacities. The effect on respiration, enzyme activity, and enhancing the growth of a viable microbial population, necessary in decomposition and mineralization not only for agronomic practices but also for several of the organic cycles—carbon and nitrogen cycling—are just a few among the indirect effects from the science of soil biological properties.

Examples of the direct effects are enhanced seed germination, rapid seedling growth, better root development and plant growth, and enhanced crop yields. With the increased knowledge in soil chemistry, all of the

effects mentioned above are now realized to be caused by the *active* inorganic and organic components. This book will focus on the agronomic significance of the organic components and, in particular, on humic matter in soils as implied in the title of this chapter. For readers also interested in the active inorganic fractions in soils, reference is made to the book *Principles of Soil Chemistry* (Tan, 2011a).

10.2 Effect on Soil Physical Properties

The physical properties of soils are noted to change due to the addition of soil organic matter yielding humic substances. These changes are usually interrelated, and the change in one physical property will often be followed by changes in other physical properties. The following explanations serve as a few examples. The soil physical characteristics of significance in agriculture are usually soil texture, soil structure, bulk density, porosity, moisture content, and more. Together with soil structure, soil texture then affects pore spaces, aeration, water flow, percolation, and leaching. Unless the concept and definitions are changed, soil texture is not known to be affected by applications of soil organic matter or humic acid, but the soil conditions will change. For example, soils with clay textures are generally sticky when wet and display high plasticity, and low porosity and permeability; hence, they demonstrate poor air and water movement. The addition of soil organic matter is not going to change the clayey texture, but will normally improve soil porosity, permeability, and aeration, transforming at the same time the high plasticity into friable conditions. Plasticity is closely related to soil texture, but belongs to the category of *soil consistency* as is friability. On the other hand, soils with sandy textures are often structureless and become rapidly dry, due to rapid loss of moisture because of a high percolation rate and due to their low adsorption capacity for water. Their adsorption capacity is usually increased significantly and the formation of soil structure enhanced by application of organic matter. Bulk density, another soil physical property, is a good measure of the density and porosity of soils. The concept of bulk density takes into account both the masses of the solid particles and the volume of pore spaces. Consequently, soil texture and soil structure are indirectly connected to bulk density. In summary, it is possible to conclude that soils high in organic matter usually exhibit high water-holding capacities, display more developed structures, have low bulk density values, and are often fluffy or friable in consistency. Owing to a lack of research data

in the literature and because of space limitations in this book, only some of the physical properties will be examined in more detail in the following sections.

10.2.1 Effect of Humic Matter on Soil Consistency

Soil consistency is defined as the strength with which soil particles are held together, providing to soils resistance to rupture or to forces of deformation.

It is selected here for discussion because it is a soil physical property caused by the combined effect of several other physical properties that are dependent on the forces of attraction between soil particles as influenced by soil moisture. Hence, by examining soil consistency, soil texture, and other physical properties related to issues with soil moisture, aeration and ease of cultivation or plowing are also studied.

10.2.1.1 Atterberg's Plastic and Liquid Limits

Five major types of soil consistencies are recognized in soil physics: hard, friable, plastic, sticky, and viscous (Tan, 2009; Soil Survey Staff, 1990). The water content increases from hard to viscous consistency, and each type of consistency can be distinguished again into several subtypes. For example, the sticky consistency is divided into nonsticky, slightly sticky, sticky, and very sticky. The friable consistency is the most favorable soil condition for agronomic practices. In general terms, the soil can exist in four major conditions. Depending on the moisture content, the soil can be present in a solid, semisolid, plastic, and liquid state, and in each state the soil's behavior is different. Solid soils exhibit hard consistencies, whereas semisolid soils are friable, plastic soils are sticky to plastic, and soils in the liquid state are more viscous in consistency. In soil physics, the limits of moisture content in each of the five forms of soil consistencies above are referred to as the *Atterberg limits*. For example, the change from the semisolid to plastic state and from the plastic to liquid state is determined by changes in soil moisture contents, as expressed by the so-called plastic limit and liquid limit, respectively (Tan, 2009). The *plastic limit* is defined as the moisture content at which the soil starts to exhibit pliability, which is determined when a thread is formed by rolling out a sample of soil. Sands have practically a plastic limit of zero. On the other hand, the liquid limit is the moisture content at which the soil starts to flow like a liquid. In general, soils high in clay contents are high in both liquid and plastic limits. The difference between the liquid and plastic

limits defines the Atterberg *plasticity index*. The liquid limit of soil is noted, from investigations by Yunus et al. (2011), to decrease with increasing humic acid content, whereas the plastic limit increases at the start of humic acid application and remains constant with further additions of humic acids. This trend for the plastic limit is clearly shown by the parabolic curve presented by the above authors in Figure 1 of their journal article.

10.2.1.2 Agronomic and Environmental Significance

Soil consistency is, perhaps, next to soil structure, one of the physical properties affecting most agronomic and engineering practices in soils. The ease of cultivating soils for growing crops is affected by soil plasticity. Plowing soils at the plastic limit usually destroys the soil structure, and upon drying often cakes the soil or may result in crust formation in soil surfaces. However, soil consistency also plays a significant role in environmental issues. The term plasticity finds its roots in the pottery industry some 100 years ago. High-plasticity soils, similar to soil used in pottery, turn into sticky mud when mixed with water. Therefore, heavy rains saturating high-plasticity clayey soils located on steep slopes tend to turn the soils into the liquid state, resulting in landslides. These soils need a protective ground cover to prevent landslides and further erosion. However, some crops, e.g., paddy rice, require soil conditions in the liquid state for proper growth and yield.

10.2.2 Effect of Humic Matter on Soil Structure

Although all of the physical properties are no doubt important for the well-being of the soil ecosystem, it is perhaps soil structure that is the most significant in soil formation and soil degradation, as well as in plant growth and environmental quality. The formation of soil structure favorable for plant growth is assumed to be caused by the interaction between humic acid and clays and/or by complex reactions between humic acid and Al and other metal ions. Since soil structure also infers a mutual arrangement of the three soil phases—solid, liquid, and gas—a change in soil structure will affect the balance between these three phases. The liquid and gas phases are especially vulnerable since they are also subject to continuous exchanges with the environment. The effect of humic acid is to create and preserve a stable structure that can provide the proper amounts of pore spaces for the storage of optimum amounts of water and oxygen for plant growth. The presence of durable soil structures are also of utmost importance in environmental

issues, because well-structured soils are noted to be less prone to erosion and other harmful denudation processes.

10.2.2.1 Self-Assemblage or Traditional Aggregation Concept

With the introduction of the micellar and supramolecular concepts in humic acid chemistry (see Chapter 3), the present author introduces the idea of adapting the theory of self-assemblage also in the formation of soil structure. Many similarities seem to be present between the self-assembly process of humic substances and the spontaneous aggregation and disaggregation processes in soil structure formation. The concept has been examined in some detail in Chapter 3, Section 3.5.1, where the issue is underscored by offering a unique example in paddy soils. The surface of these soils is puddled to reach the liquid limit needed for lowland rice cultivation under inundated (irrigated) conditions. When after the rice harvest, the structureless soil is left to recover naturally, the surface soil appears to regain its original structure. The recovery process is noted to yield granular or crumb structures by a regrowth of a vegetative cover. Therefore, the author believes that spontaneous self-assemblage of soil materials is more common in nature than would have been expected, and is not an isolated process occurring only in the formation of humic substances. The new concept of self-assembly in soil structure formation is not opposing but is blending in with the traditional older aggregation theory. The existing idea of humic substances serving as cementing agents and other natural effects on the formation of soil structure is compatible with the new theory based on supramolecular principles of self-assemblage. An additional advantage is that the new proposed self-assembly theory takes into account formation of soil structure both at the macroscale and microscale, whereas the older aggregation concept is more on the macroscale. The suggested self-assemblage idea now provides the scientific basis for the concept of microstructures, recognized in soil physics to also coexist with macrostructures. The significance is now also the advantage of the humo-nanotube concept, bringing a capability of tailoring the investigations on soil structure exactly at the nanoscale and microscale where the basic properties and phenomena are initiated. It is structure formation at a very small scale and belongs to a section of science previously called *nanoscience*, as discussed in some detail in Chapter 3. This new generation of science includes the use of functional structures, designed from an atomic scope with at least one specific dimension measured in nanometers. This tiny size is responsible for the unique physical property, such as the

soil microstructure, as well as for its related processes of formation and various phenomena.

10.2.2.2 Cementation and Crust Formation

The cementation effect of humic acid has long been considered a major factor in the formation of soil structure (Baver, 1963), which is important especially in sandy soils. These soils are usually very loose and friable, and often single grained or structureless. The amount of clay present is insignificant in cementing the sand particles together. The dominant amounts of sand particles, which are chemically inert, are incapable of interacting between themselves or with either cations or water. Consequently, the assemblages formed by spontaneous self-aggregation of sand particles are expected to be stable only by cementation with humic acid. In contrast, another problem arises in soils low in sand but rich in clay and silt. Here, crust formation may occur upon drying when the soils are also low in organic matter contents. Clays exist in the form of tiny thin plates or sheets, which upon drying may settle closely on top of one another like roof shingles. For farmers, the occurrence of surface crusts provides a big problem. Seed germination, soil aeration, and infiltration of water will be inhibited. Enhancing aggregation by adding soil organic matter is often noted to prevent the formation of these surface crusts. The absence of soil organic matter in clayey soils causes, in addition, the development of structureless conditions, and the so-called massive structures commonly formed will inhibit aeration, water penetration, and root growth. By adding humic acids, the interaction of clay with the organic compounds may result in the formation of crumb and granular structures, alleviating the unfavorable physical conditions for plant growth and crop production.

10.2.2.3 Chelation and Bridging in Structure Formation

Although its cementation effect plays an important role, the interaction of humic acid with metal ions and clays is believed to be a more decisive factor in many soils for the creation of stable soil structures. One of the most striking examples in this respect is the unique physical condition in andosols. These soils are known for their black color due to high organic matter contents, low bulk densities, and crumb to granular structures. The soils can become very wet due to their extremely high water-holding capacities. However, in such wet conditions, they still display low plasticity and

stickiness, are friable in consistency, and do not show signs of being in a reduced state (Tan, 1998; Shoji et al., 1993). The consensus is that with their high organic matter content, allophane and Al in andosols, have played a major role in the development of soil's unique physical properties. Although several hypotheses can be presented, it is commonly assumed that the amorphous clay and humic acids are responsible for the exceptional physical properties. Exposed groups of Al and Si on the surfaces of allophane and imogolite are capable of interacting with humic acids, forming humo-Al-allophane or humo-Si-imogolite complexes or chelates. The structural Al acts, in essence, as a connecting bridge; hence, the interaction can be called *Al bridging*. As discussed in Chapter 9, Al ions in the soil solution can react in the same way acting as bridges between humic acids and negatively charged surfaces of the clay mineral (Figure 9.6). Not only will the organic substances be protected from rapid decomposition by such an interaction, but also these chelates constitute the nucleus for the formation of granular or crumb structures. In turn, crumb structures provide for an abundant amount of macropore and micropore spaces, which together with those present in the amorphous clays account for the high total porosity exhibited by andosols. The accumulation of organic matter (humic acids), known for its high water adsorption capacity, together with the increased amounts of pore spaces therefore increases the water-holding capacity of the andosols.

A similar soil physical development can also be noticed in mollisols. However, the difference is that mollisols are neutral soils containing high amounts of Ca and crystalline clays, assisting in the accumulation of organic matter and the creation of excellent physical conditions. The clay fraction of these soils is characterized by smectite with kaolinite as an admixture. Smectite, the dominant clay mineral, does not have surfaces containing exposed Al hydroxyl groups. The planar surfaces are composed of siloxane surfaces and are mostly negatively charged due to isomorphous substitution. Consequently, smectite can only be attracted to humic acid with Ca ions acting as bridges (Figure 9.6). On the other hand, the kaolinite minerals present, have Al octahedrons present on side only, surfaces composed of Al octahedrons. However, these surfaces are usually negatively charged due to dissociation of the exposed hydroxyl groups. Hence, the interaction of kaolinite with humic acid is made possible only through the Ca-bridging mechanism. The humo-Ca-clay mineral chelates are considered the reasons for the preservation of soil organic matter (humic acids) and for the development of granular structures and other favorable physical characteristics in mollisols. Soils containing smectite in their clay fraction often experience

a common problem, manifested in the formation of large cracks when dry. This is known to be caused by the high swelling and shrinking capacity of the expanding 2:1 type of clay minerals, such as smectite. The large cracks formed modify the soil's behavior with respect to aeration and water penetration, and may cause damage to plant roots. The effect of humic acid, as discussed above, by interacting with the expanding clay is noted in mollisols to reduce the extent of shrinking and swelling.

10.3 Effect on Soil Chemical Properties

10.3.1 Charge Formation by Functional Groups and pH

Humic matter can affect the soil chemical properties in various ways since it can generate a variety of chemical reactions. As indicated before, the chemical behavior of humic matter is, in general, controlled by two functional groups: the carboxyl and phenolic-OH groups. The carboxyl groups start to dissociate their protons at pH 3.0 (Posner, 1964), and the humic molecule becomes negatively charged (Figure 9.1). At pH <3.0, the charge is very small or even zero. At pH 9.0, the phenolic-OH groups also dissociate their protons and the humic molecule attains a high negative charge. The issue of these pH values in the dissociation of functional groups of humic substances in natural soils has been discussed in Chapter 9.

Since the development of the negative charge is pH dependent, this charge is called *pH-dependent charge* or *variable charge* (Tan, 1998). A number of reactions can take place because of the presence of these charges. At low pH values, the humic molecule is capable of attracting cations, and such electrostatic attraction leads to cation exchange reactions. This kind of reaction will no doubt affect the CEC in soils. The CEC of humic matter can be estimated from its total acidity values, which are usually very high. Humic acid shows CEC values, in terms of total acidity values, ranging from 500 to 1200 cmol/kg, whereas fulvic acid exhibits a somewhat higher range of 600 to 1500 cmol/kg (Tan, 1998, 2000; Schnitzer and Khan, 1972). At high pH values, when the phenolic-OH groups are also dissociated, complex reactions and chelation become of importance (Figure 9.2). Complex reactions are considered to be a weaker bonding mechanism than chelation owing to the formation of a coordinate bond with a single donor group. On the other hand, chelation is viewed to be a stronger bonding process because of the formation of a chelate ring structure by more than one donor group. Both

adsorption and complex reactions can also take place by a water- or metal-bridging process. This was the vehicle for interaction reactions between humic matter and clay as discussed earlier.

10.3.2 Functional Groups in Chelation and Adsorption as Affected by Soil pH and pK

Some scientists assume that interactions with metals are the most important processes and are going to occur first at sites that form the strongest bonds, e.g., coordinate bonding and chelating sites. As these stronger bonding sites become saturated, attraction to the weaker sites, e.g., electrostatic-bonding and water-bridging sites, become increasingly greater (Stevenson, 1994).

However, the present author believes that under normal soil conditions, the above assumption is difficult to justify. At low pH or pH values normally occurring in agricultural soils, the only sites available are the sites for electrostatic attraction and the sites for water bridging. The COOH functional groups are 50% dissociated (deprotonated) at $\text{pH} = \text{pK}_1 = 3.0$, but instead are fully charged at $\text{pH} = 5.0$, as examined in Section 9.1.2.3. In contrast, neither the complexing nor the chelation sites are ready for reactions because, at that pH value, the phenolic-OH functional groups are practically fully protonated or nondissociated at all. As calculated with the Henderson-Hasselbalch equation, the dissociation of the phenolic-OH groups will amount to 99% at $\text{pH} 11.0$, a pH value too high for soils to be agriculturally productive.

10.3.3 Role of Humic Matter in Chelation Affecting the Environment

Nevertheless, the complexing and chelation capacity of humic matter is considered today of utmost importance in many environmental quality issues. Depending on several factors, e.g., pH, degree or rate of saturation of sites, and electrolyte concentration, humic matter can form both soluble and insoluble complexes with metals, hence providing for a dual function in the soil ecosystem. In natural conditions, most of the chelates may be in insoluble forms due, in part, to the participation of clays in the reaction process. The fulvic acid fraction is assumed to form the more soluble metal chelates because this humic substance is soluble in water to begin with, is lower in molecular weight, and has higher contents of functional groups. The fulvo-metal chelates remaining soluble may then serve as carriers of trace metal

elements to be transported to plant roots. On the other hand, humic acid tends to produce more insoluble metal chelates, and the humo–metal chelate is considered to serve as a *sink* for toxic metals. Large amounts of free Al in acid soils are made chemically inactive by chelation with humic acid, preventing Al toxicity in crops and plant growth (Tan and Binger, 1986). Hence, humic acid can act as a buffer in alleviating the adverse effects of heavy metals and toxic substances such as pesticides and other xenobiotics. However, depending on many factors, such as the type of metallic ion, cationic valences, cation saturation, and degree of dissociation of the humic molecule, humic acid is also capable of forming soluble metal chelates. This is considered of extreme importance by many chemists and environmentalists in the mobilization and concentration of radionuclides in the environment (Gaffney et al., 1996a). The chelates, carrying the toxic compounds, can migrate long distances and may pollute the groundwater or reappear at other locations to be precipitated. Nonpolar hydrophobic compounds, e.g., dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB), can be made soluble in this way, hence preventing their accumulation in soils and sediments. However, the creation of insoluble and soluble chelates by humic acids seems to generate controversial problems for the environment. In the form of humo–chelates, these xenobiotics are indeed prevented from adsorption by soil clays; however, the interaction with humic acids decreases their rate of decomposition, photolysis, volatilization, and biological uptake. The latter is expected to lengthen their lifetimes or to increase their mean residence time. This is expected to also affect their transport distances in our natural ecosystem.

10.4 Effect on the Soil Redox System

10.4.1 Humic Substances as Electron Donors

The reduction and oxidation reactions in soils, called *redox reactions*, are chemical processes involving electron transfer. They affect the formation and accumulation of humic matter. As explained earlier, more humic matter will be formed in reduced than in oxidized environments, as exemplified by formation of peats. However, it is also noted that humic matter is capable of inducing reduction and oxidation reactions, hence affecting the redox system in the environment. Humic substances are in fact important components of the soil redox systems, capable of transferring electrons (Flaig, 1975, 1988).

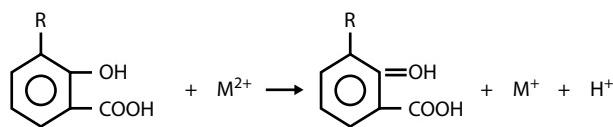


Figure 10.1 Schematic representation of a redox reaction of humic acid showing electron transfer to a metal M^{2+} .

They are considered by Ziechmann (1994) *electron donor–acceptor complexes*, with the aromatic structures, containing OH and COOH groups, functioning as electron donors, and quinonoid structures as electron acceptors. However, most of the data presented thus far are based on the capacity of humic substances as electron donors, with the transition metals at the higher oxidation states serving as the electron acceptors. A reaction of such an electron transfer is illustrated in Figure 10.1, by which a divalent ion (M^{2+}) is reduced into a monovalent ion by accepting an electron from the humic acid molecule.

10.4.2 Redox Potential and pe Concept of Humic Matter

In reduction–oxidation chemistry, compounds capable of donating electrons are sometimes referred to as electron-rich substances embodied by substances in the reduced state, whereas their counterparts, compounds capable of accepting electrons, are called electron-poor substances, which are the materials in the oxidized state (Tan, 1998, 2011; Sposito, 1989). Electron-rich substances are usually characterized by negative pe values, whereas the electron-poor compounds commonly exhibit positive pe values.

The parameter pe is often used for measuring the capacity of substances to donate or accept electrons (Tan, 1998, 2011). It is derived from a generalized redox equation as follows:



for which the electrochemical potential, measured against a standard hydrogen electrode, E_H , is defined as

$$E_H = E^\circ + \frac{RT}{nF} \ln \frac{\text{Oxidation}}{\text{Reduction}} \quad (10.2)$$

in which E° = standard electrochemical potential, R = gas constant, T = absolute temperature (°Kelvin), n = valence, and F = Faraday constant.

Since the reaction is a reduction–oxidation reaction, the electrochemical potential, E_H , is called a *redox potential*. In the above equations, the term oxidation refers to substances in the oxidized state and the term reduction refers to substances in the reduced state. The oxidized materials carry higher valences than their reduced species, and the electrons have the responsibility of balancing the equation. In the case above (see Equation 10.1), the oxidized substance is one valence higher than the reduced element, which carries one lower valence due to reaction with the electron.

If electron (e^-) activity increases, the reaction shifts to the right, meaning reduction takes place. When, on the other hand, electron activity decreases (no e^- available), the reaction shifts to the left, or in other words oxidation occurs. In analogy to the concept of pH, electron activity can be represented by pe as illustrated below (Tan, 1998, 2011):

$$pH = -\log (H^+) \quad (10.3)$$

$$pe = -\log (e^-) \quad (10.4)$$

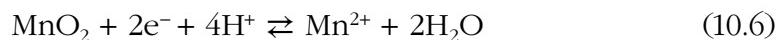
It should be realized that the analogy also includes the fact that neither electrons nor hydrogen ions can exist as free particles in natural conditions or in the soil solution. Both can exist only in association with the solvent or a solute species.

The parameter pe is closely related to the redox potential E_H according to the following equation (Tan, 1998, 2011):

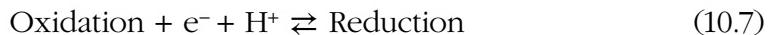
$$E_H = 0.059 \text{ pe} \text{ or } pe = E_H / 0.059 \quad (10.5)$$

From the above, it follows that pe can also represent the redox potential since conversion of E_H into pe (or vice versa) can be accomplished very easily by using Equation 10.5. However, it should be realized that pe was not defined as a redox potential but is, by definition, the activity of electrons (see Equation 10.4). The redox potential is the electrochemical potential (E_H) of the redox reaction and has been defined as reflected in Equation 10.2.

Electron transfer in redox reactions involving oxides is sometimes written accompanied by a proton (H^+) transfer, as illustrated by the reaction below



This leads some scientists to believe that a redox reaction can accordingly be generalized as suggested below by Bartlett (1999):



Unfortunately, generalizing a redox reaction with the inclusion of a proton or H^+ ion as written above creates an unbalanced equation. The error is clearly noticed in Equation 10.8 when the real substances are used, e.g., oxidation is substituted for Fe^{3+} and reduction for Fe^{2+} :



Treating the generalized redox reaction 10.7 as a dissociation reaction to determine the redox potential is also confusing the basics of electrochemistry. The dissociation or equilibrium constant k is written by Bartlett (1999) as follows:

$$\log k = \log \text{red} - \log \text{ox} - \log e^- - \log H^+$$

or

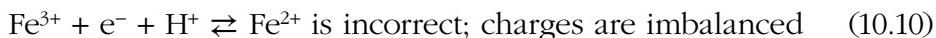
$$\log k = pe + pH \quad (10.9)$$

in which $\log k$ = the log of the dissociation constant k .

$\log k$ equals ($pe + pH$) is possible only when $(\log \text{red} - \log \text{ox}) = 0$, whereas considering $pe + pH$ as the redox parameter or redox potential cannot be justified by Equation 10.9. This is stretching the basics of electrochemical potentials a little bit too far. According to Equation 10.8, $pe + pH$ equals the logarithm of the dissociation or equilibrium constant. Treating the reaction as a dissociation process is perfectly normal; however, an equilibrium constant is then found and not an electrochemical potential of a redox reaction. The electrochemical potential of a redox reaction can be formulated only by treating reaction 10.7 as a redox process, and the redox potential, E_H , can then be formulated as shown in Equation 10.2.

The electrons are also added in redox equations for the purpose of balancing the equations by reducing the charges of the oxidized substance. Hydrogen (H^+) ions are not added in generalized redox reactions as shown in reaction 10.7 and, as stated before, it results in an unbalanced reaction equation. The hydrogen ions added must be accounted for and are used for

convenience to convert the oxygen (O_2) atoms into H_2O , as noted in reaction 10.6. Additional examples are presented below to illustrate more clearly the issue of incompatibility of Equation 10.7 for application with real ionic components:

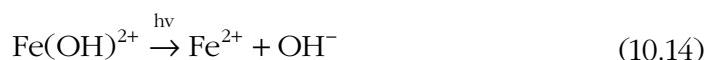


In soil chemistry, standard half-cell reactions involving electron transfer between ionic components are written in accordance with the International Union of Pure and Applied Chemistry without the hydrogen ions (Weast, 1971). This is the process called electron transfer, and the number of electrons transferred is used for the determination of equivalencies or equivalent weights.

10.4.3 Concept of Reduced–Oxidized Forms of Humic Acids

As discussed above, electron availability can be used as an indication of the reduction–oxidation status of the soils. It affects the oxidation and reduction states of H, C, N, O, S, Fe, Mn, Cu, Zn, and many other elements, and therefore controls the solubility and availability of many nutrient elements to plants. Ions in the reduced forms are usually more soluble, hence more available, to plant roots.

Oxidation–reduction reactions of metal ions can occur in soils in several ways. They can be mediated by microorganisms, induced by the presence of organic compounds such as humic acids, and induced by photochemical reaction. Ultraviolet radiation at a wavelength between 360 and 450 nm is believed to be able to reduce iron. The process, called *photoreduction*, can be illustrated by the following reaction (McKnight et al., 2001; David and David, 1976):



in which $h\nu$ is the energy of radiation required for the reaction. The reaction is apparently reversible since the authors claim that the OH radical can reoxidize Fe^{2+} back into Fe^{3+} . The present author wonders only about the OH group dissociated as an uncharged ion, which raises many questions and hence is highly controversial.

Insoluble Fe(III) oxides can also be reduced into Fe(II) oxides by photochemical reduction. The process is allegedly mediated by the presence of humic substances in the reduced state, which will be photo-oxidized into oxidized humic substances. The reactions are written by Sulzberger et al. (1994) and Sulzberger and Laubscher (1995) as follows:

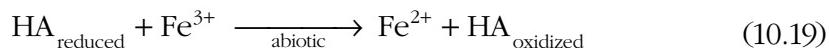


The release of the Fe^{2+} as an ion (Equation 10.16) is called by the authors a *detachment* process. In soil chemistry, we call this *dissolution*. The rate of reaction 10.16 versus that of reaction 10.17 is considered by the authors of importance for the overall *quantum yield* of photoreduction. They believe that this quantum yield, in moles of Fe^{2+} produced per photon of incident UV radiation, is higher for dissolved Fe(III) species than for Fe(III) in the oxide form. Increasing degree of crystallinity of Fe oxides is assumed to decrease the quantum yield since amorphous iron oxides are noted to be substantially more photoreactive than the crystalline goethite or lepidocrocite minerals. The quantum yield also decreases with increasing soil pH because Fe^{2+} is unstable and cannot exist at a high soil pH. It tends to be oxidized and precipitated rapidly into Fe(OH)_3 in alkaline soils.

The photochemical dissolution of reduced iron is assumed by McKnight et al. (2001) to be a temporally dynamic process, causing its concentration to fluctuate. These authors also claim that sorption of humic substances by Fe oxides enhances the photoreactivity of recently precipitated oxides, but that such sorption decreases the photoreactivity of aged iron oxides. The latter seems to be in support of the effect of increasing crystallinity on photoreactivity of these iron compounds as discussed above.

This process of photoreduction is, of course, an interesting topic. However, the terms *reduced HA* and *oxidized HA* are mystifying and perhaps

misplaced. Neither chemically nor analytically humic acids in reduced and oxidized forms are noted to exist naturally, even in the sense of operational substances. Their presence is controversial and subject to many arguments. Lately several chemists seem to advance the idea about the role of humic substances in redox reactions with metals through transformation of humic matter from a reduced into an oxidized state. The conversion process is presumably mediated by microorganisms, and the reactions can perhaps be written as follows (Lovley et al., 1996, 1998):



No further explanations are provided by Lovley and coworkers, leaving the present author to provide the following reasons. Reaction 10.19 indicates that the humic substance labeled $\text{HA}_{\text{reduced}}$ is an electron donor, and Fe^{3+} is then the electron acceptor. This reduced humic substance therefore has excess electrons and is then negatively charged because, by the rules of soil chemistry, compounds with excess electrons are negative in charge. The substance labeled $\text{HA}_{\text{oxidized}}$ must be a neutral or noncharged humic substance since only in this way can Equation 10.19 be balanced with 2^+ charges on both sides.

Although it seems to be a legitimate reaction, Equation 10.19 nevertheless creates a lot of questions. Equation 10.18 indicates the production of CO_2 , which means that a carbon is lost during the microbially induced electron transfer to the oxidized form of HA. The reaction as written is misleading since the CO_2 can be interpreted as being derived from the decomposition of the oxidized HA compound by the microbes. That this is far from true can be seen from the following explanations gathered by the current author. The iron-reducing microorganisms in freshwater and marine environments are believed to have the ability to use humic matter as electron acceptors (Coates et al., 1998). This opinion is used by Lovley et al. (1996) for advancing their idea that in anoxic conditions, humic substances can accept electrons released by microbial oxidation of other organic substrates. The latter then accounts for the CO_2 in the equation, which in fact does not participate at all in the reaction as written in Equation 10.19, and deleting the CO_2 will not change the balance of the reaction as written. By donating the electrons to Fe^{3+} , the humic molecule is considered to be reoxidized.

The theory above, although interesting, is contrary to the electron donor-acceptor concept presented by Ziechmann (1994) and Stevenson (1994). As indicated earlier, these authors argue that the aromatic structures containing phenolic-OH and carboxyl groups are functioning as electron donors. These functional groups are, by nature, protonated; hence, they represent reduced conditions. When these functional groups become negatively charged, they are carrying, in other words, excess electrons. On the other hand, the quinoid or quinone units in a humic molecule are usually assumed to be phenol structures in the oxidized state (Schubert, 1965; Flaig et al., 1975; Stevenson, 1994). Humic molecules containing these functional groups then represent humic substances in the oxidized state. However, since they are also considered to be electron acceptors (Ziechmann, 1994), it is rather confusing how reaction 10.18 can take place yielding two types of electron acceptors, e.g., Fe^{2+} and oxidized humic acid, or $\text{HA}_{\text{oxidized}}$. The reaction is then unbalanced, unless the term $\text{HA}_{\text{oxidized}}$ is referring to neutral humic substances as indicated before. However, neutral humic acids are only present when fully protonated; in other words, in “reduced” form. As indicated above, it is a highly controversial issue and, by accounts of Palmer and von Wandruszka (2010), the functionalities of humic substances responsible for the redox reactions remain in question and, as of today, are still not fully understood.

In the presence of humic substances, a similar reduction process has been reported for MoO_4^{2-} to Mo^{5+} (Lakatos et al., 1977; Goodman and Cheshire, 1972; Skogerboe and Wilson, 1981). This, in addition, supports the idea that humic substances exhibit high negative pe values. Insoluble Mn oxide (MnO_2) is noted to also be converted into soluble Mn^{2+} by marine humic acid and fulvic acid (Harvey and Boran, 1985). The conversion of insoluble Mn into the soluble form is likewise assumed to be a photoreduction process and considered by the authors of high biological importance in the aquatic environment. Manganese is an essential nutrient for marine plankton. Since marine humic acid is the ultimate reducing agent, the formation of humic acid is viewed by Harvey and Boran (1985) as an ecological feedback.

10.5 Effect on Soil Biological Properties

Humic substances are considered energy-rich material and play an important role in plant and microbial growth and the biochemical cycles in soils. They have been formed from phytogenic, animal, and microbial substances, and

as such are assumed to be relatively stable, at least more stable than carbohydrates or protein. However, since they still contain a lot of energy, the humic compounds are subject to further decomposition and will eventually be broken down by soil organisms into CO_2 and H_2O .

A multitude of interactions between humic matter and soil biota are present in the literature. The most extensive reports have probably been on its effect on plant growth and crop production. This will be the topic of the next section. Less known is the effect of humic matter on microorganisms and biochemical processes, which according to the little information available in the literature can be distinguished into an indirect and a direct effect. Changes brought about by humic matter on biochemical chemical processes may have a pronounced effect on microbial development and activity; hence, such an effect can be considered an indirect effect. Two examples of importance are the effect of humic matter on the carbon and nitrogen cycles.

10.5.1 Carbon Cycle

The carbon cycle is the perpetual movement of organic carbon from the air into the soil and back into the air. It is nature's way of cleaning the environment by recycling organic waste (Tan, 2000). In principle, the cycle starts when CO_2 gas in the atmosphere is absorbed by green plants and converted into carbohydrates by a process called photosynthesis. With leaf fall and when plants die, the vegetative remains are subject to decomposition and mineralization processes, which return the carbon from the soil to the air as CO_2 gas. For more detailed information on decomposition and mineralization, two important biochemical processes assisting the cycle to run, reference is made to Tan (2000, 2011) and Stevenson (1986).

Humic matter plays an active role in the fixation and release of organic carbon. By fixation of part of the organic carbon in the form of humic substances, this process is conserving the organic carbon and at the same time reducing the production of CO_2 . Humic acids have a carbon content of 50% to 57%, but most of it is relatively more resistant to microbial attack than that of carbohydrates. This carbon reserve in humic matter worldwide was assumed earlier to amount to 10^{12} metric tons; however, it is believed that most of it is unavailable as a direct carbon source for many of the microorganisms in soils (Müller-Wegener, 1988). The resistance of humic matter to biological decomposition has been expressed by Stevenson (1994) in terms of mean residence time (MRT), which is the age of humic matter from the time of formation to its decomposition in soils. Although MRT values seem

to vary considerably from 250 to 1900 years, they nevertheless indicate the relative stability of humic substances to microbial attack. It should be realized that MRT does not represent the absolute age of humic substances, but reflects only the average age because of the transient nature of the compounds in soils, where "old" humic matter is continuously decomposing while "new" humic material is synthesized. Decomposition of humic matter is also expected to be more rapid in aerobic than in anaerobic environments. Under well-aerated conditions where oxygen is not a limiting factor, oxidative degradation processes are more likely to occur, producing CO₂ and H₂O to complete the interrupted carbon cycle. Nitrogen and sulfur compounds are released as by-products (Stevenson, 1986). The final stage of decomposition is expected to be the breakdown of the more resistant lignoid or phenol part of the humic molecule, in which actinomycetes and fungi are considered to play a major role. In simulated oxidative degradation analyses of humic acids, fatty acids, aliphatic carboxylic acids, phenolic acids, and benzene carboxylic acids are produced (Griffith and Schnitzer, 1989). However, whether similar compounds are produced in the natural process remains a question for argument.

In poorly drained soils and soils of the wetlands, decomposition reactions of humic matter occur at a greatly reduced rate. These soils support a different population of microorganisms that produce different types of end products, although CO₂ is included for continuation of the carbon cycle. An incomplete decomposition under anaerobic conditions generally yields fermentation products, e.g., methane, mercaptans, nitrosamines, and the like, some of which are foul smelling whereas others are believed to be carcinogenic (Stevenson, 1994). The main decomposition processes in such environments are expected to be hydrolysis and reductive cleavage. A schematic representation of the formation of methane through hydrolysis of humic substances by methanogenic bacteria is given below as an example:



Whether the processes in a natural environment can be simulated under laboratory conditions is again an issue open to question. Hydrolysis of humic matter in nature is an enzymatic reaction, whereas in the laboratory the reaction is catalyzed by acids or bases, which according to Parsons (1989) involves the cleavage of bonds. However, it seems that only simple and peripheral bonds are broken down (Figure 10.2). The more complex bonds,

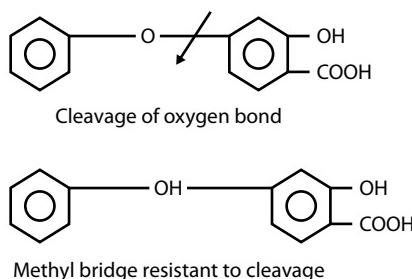


Figure 10.2 Decomposition of humic matter by reductive cleavage of aromatic bonds using the Na amalgam procedure. (From Piper, T. J., and A. M. Posner, *Plant and Soil*, 36, 595–598, 1972; Stevenson, F. J.: Reductive cleavage of humic substances. In: *Humic Substances II. In Search of Structure*. 1989. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

such as aromatic bonds through methylene bridging, have been reported to be very resistant as noted in the analysis by Piper and Posner (1972) and Stevenson (1989) using the reductive Na amalgam method.

In summary, it can be stated that humic matter is an active constituent of the organic cycle in the soil ecosystem. Utilizing organic carbon for the formation of humic substances means preserving it for the benefit of the physico-chemical condition of the soil ecosystem. It is a form of soil carbon sequestration, a process considered of vital importance for the environment (Lal, 2001). Although relatively stable, the stored carbon remains a formidable energy source for many microorganisms. The microbial population is often noted to thrive prolifically in soils rich in humus. By way of enzymatic decomposition and mineralization, the humic substances are eventually broken down into H_2O and CO_2 , which completes the cycle.

10.5.2 Nitrogen Cycle

The nitrogen cycle is another indirect effect of humic matter on the biological properties of soils. Simply defined, it is the movement of nitrogen from the atmosphere through the plants into the soil, before it is returned to the atmosphere in its original gaseous state. The cycle is composed of a sequence of biochemical reactions involving the active participation of the soil microbial population and plant life. For more details on the specific biochemical reactions, reference is made to Tan (2000, 2011) and Stevenson (1986). As illustrated in Figure 10.3, the nitrogen cycle involves an *outer cycle* representing the overall cycle, and an *inner cycle* that occurs in the soils. In

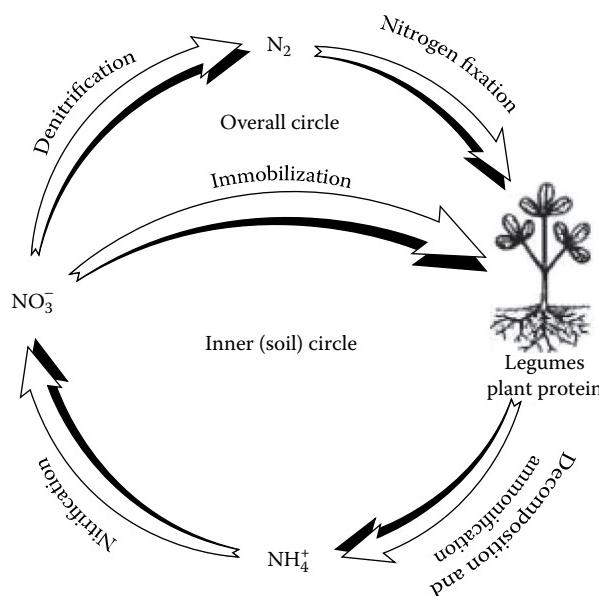


Figure 10.3 Simplified diagram of the nitrogen cycle, showing the overall and the inner cycles. (Drawing by W. G. Reeves, art coordinator, University of Georgia.)

the inner cycle, the NO_3^- is not denitrified, but consumed by plants and soil organisms by a process called *immobilization*. The cycle is completed in the soil, and it is here that humic matter plays an active role in affecting the nitrogen cycle. As a result of decomposition of plant residues, a variety of nitrogenous compounds are released, e.g., amino acids, amines, and peptides, part of which are used in the synthesis of humic matter, leading some authors to regard this as an immobilization process (Müller-Wegener, 1988). However, *nitrogen fixation* is perhaps a better term, although the mechanism is not similar to NH_4^+ fixation by expanding clays. It is more the incorporation of nitrogenous compounds in the humic molecule, which perhaps can be likened to N-fixation by nitrogen-fixing bacteria, with the difference that gaseous N is utilized by the microorganisms, whereas solid or ionic compounds are involved in the synthesis of humic substances. Because of this, the N content of the humic molecule tends to increase, affecting its carbon-to-nitrogen (C/N) ratio. Generally the C/N ratio of plant residues varies from 80:1 in wheat straw to 20:1 in legume material (Tan, 2000; Stevenson, 1994). While, on the one hand, part of the organic carbon is incorporated in the humic molecule, with the remainder being lost as CO_2 in the decomposition process, on the other hand, N is being added into the humic structure. These processes cause the C/N ratio of the humic substances to decline to

the narrowest ratio at which C and N can exist together in soils. Generally the C/N ratio of humic matter may fall to relatively stable values. A carbon content of 50% to 57% and a nitrogen content of 4% to 5%, giving a C/N ratio of 10:14, are characteristic of well-developed humic acids. The nitrogen stored in the humic molecule will be released again after decomposition and mineralization of the humic substances. Part of it will be used by the micro-organisms, whereas the remainder will be subject to ammonification and nitrification processes in continuation of the nitrogen cycle.

10.5.3 Fixation of Agrochemicals

This is another indirect effect of humic matter on soil biological properties. With the agricultural revolution, increasing amounts of inorganic and organic compounds are introduced in the soils as wastes. Most of them will affect the flora and fauna in the soil ecosystem, some of them beneficially but many others harmfully. Humic matter with its huge cation exchange and chelation capacity can adsorb and detoxify a number of toxic compounds. Reduction of micronutrient toxicity, most resulting from heavy metals such as Fe, Cu, Zn, and Mn, has been presented earlier by underscoring the alleviation of Al toxicity in plant growth by humic acids (Tan and Binger, 1986; Ahmad and Tan, 1986). From an environmental or ecological standpoint, the chelation of heavy metals by humic substances may also reduce toxic hazards for human beings and animals. This is especially important in view of the huge production and disposal of large amounts of domestic and industrial sewage sludge, notorious for their extremely high contents of heavy metals (Tan, 2000). The adsorption and chelation of these toxic metals by humic acids represent important processes of detoxification.

Of considerable interest in this respect is also the interaction of humic substances with pesticides and their degradation products. In the aforementioned section, the possibility has been raised that pesticide residues may form stable complexes with humic matter. Such an interaction may, on the one hand, greatly increase their persistence in soils, but on the other hand may also reduce their activity. Two interactions are suggested to play major roles in the controversial effects: (1) direct linkage of the pesticide to the humic molecule, allowing the preservation of the active sites of the pesticides, and (2) synthesis of humic acid-like compounds using pesticide residues, which brings about the inactivation of the pesticides. Direct linkages occur, for example, when basic pesticides, such as *s*-triazine, react with the carbonyl groups of humic acids (Stevenson, 1994), and pesticides containing

carbonyl groups react with amino groups of humic acids. Cross-coupling of xenobiotics with humic acids is another possibility. The biodegradation of pesticide residues, on the other hand, yields reactive products that can link with carbonyl, carboxyl, phenolic-OH, and amino groups of the humic molecule to form new humic acid-like substances. Such a process usually results in a deactivation of reactive sites. Because of its incorporation, building the molecular structure of a new humic substance, the identity and behavior of the pesticide have been completely erased. The importance of such a reaction in environmental quality is without question, and many express the opinion that fixation of environmentally relevant xenobiotics by humic acids will have a positive effect on plant and microbial life.

10.5.4 Effect on Enzyme Activity

This effect of humic acids has only recently attracted research attention. Although almost all biochemical processes are enzymatic in nature, their complex and variable structure and most often the extreme difficulties and inability to directly determine enzymes in soils cause many soil researchers to shy away from studying the issue. Enzymes are proteinaceous compounds and can be determined indirectly through their capacity to transform one compound into another. By definition, enzymes are thermolabile catalysts produced by living tissue but capable of action outside the tissue (Gortner, 1949). Hormones, such as auxin, are excluded since they conduct their physiological functions only in the living tissue. A catalyst is then a substance capable of altering the speed of a reaction without appearing as part of the final product. The names of enzymes all end with *-ase* and are descriptive of the type of compounds broken down. For example, *cellulase* is an enzyme that breaks cellulose into its constituent sugar components, whereas *protease* is important in the splitting of protein into amino acids and *urease* breaks down urea, found in fertilizers and urine, into ammonia. Some of the enzymes are produced by plant cells as *constitutive enzymes*, whereas others are only produced when a susceptible substrate is present, and such enzymes are called *induced enzymes*. Cellulase is an example of an induced enzyme, while urease is an example of a constitutive enzyme. Free enzymes, enzymes not associated with the microbial biomass, are reported to be accumulated in soils by entrapment (fixation) within intermicellar spaces of expanding lattice clays (Paul and Clark, 1989), although the opinion in clay mineralogy is that most enzymes are too large to penetrate the intermicellar spaces of clay minerals. According to the purpose of this book, in

the following discussion the focus will be on enzyme–humic acid interactions only. For more details on clay–enzyme interactions, reference is made to Tan (1998). Complex formation and interaction between enzymes and humic matter are better reasons for the presence of free enzymes in soil humus. In the form of humo-complexes, the enzymes are protected from physical and biochemical attack, and remain stable and active. In this way, the compound ATP (adenosine triphosphate), an important coenzyme in all biosynthetic and catabolic cell reactions, can be present in soils, although ATP has not been isolated yet from soils. It is these free enzymes that make organic and nutrient cycling in soils possible. Much of the organic C and N entering the soil are compounds that are polymeric in nature, and as large organic compounds would not be available for uptake by higher plants and microorganisms, unless their molecular masses are reduced by enzymatic depolymerization. It would be an environmental disaster if enzymes would not survive in soils. Without the interaction with clay and humic matter, free enzymes tend to be denatured rapidly by a host of physico-chemical properties, e.g., pH and ionic composition, or tend to serve as substrates for proteolytic microorganisms (Burns, 1986). The possibility has also been offered that the enzymes can be incorporated in the structure of the humic molecule during the synthesis of humic matter. By doing so, humic matter is reported to modify the structure and affect the active sites of the enzymes (Müller-Wegener, 1988).

Although a lot of information is now present on soil enzymes, the effect of humic acids on their activities is still inconclusive, which is attributed to the mixed results produced by the various studies conducted on this aspect. In their investigations with a series of proteolytic enzymes, Ladd and Butler (1975) noticed that the activities of papain, subtilopeptidase A, thermolysin, and ficin were stimulated by humic acids. In contrast, humic acid decreased the activities of carboxypeptidase A, trypsin, and pronase B. The effect of humic acids on enzyme activities seems to also vary according to plant species. Whereas invertase activity has been reported to decrease in wheat roots, the activity of this enzyme seems to be stimulated in pea (*Pisum sativum*) roots (Malcolm and Vaughan, 1979).

10.5.5 Effect on Organisms

10.5.5.1 Soil Organisms

Different opinions are available in the literature on the direct effect of humic matter on the growth and activity of organisms in the environment. Present in

the soil as chemically reactive colloids, humic compounds are known to interact with inorganic and organic soil components and metal ions, thus modifying soil conditions for plant growth. Burges and Latter (1960) and Prát (1960) believe that humic acid is a source of food and energy for microorganisms. This is supported by Mathur and Paul (1966), who indicate that *Pseudomonas sinuosa*, *Actinomyces* sp., and other bacteria can use humic acid as a source of C and N. Neelakantan et al. (1970) have reported similar findings with *Aspergillus* sp. and *Streptomyces* sp. Using ¹⁵N-labeled humic acid, Andreyuk et al. (1973) found humic acid to be a source of N for *Bacillus megaterium*, *Pseudomonas fluorescens*, *Actinomyces globisporus*, and *Mycobacterium citreum*. By utilizing humic C and N, the microorganisms apparently decompose the humic acid, since Bardway and Gaur (1972) notice absorption of humic acid by *Rhizobium* sp. and *Azotobacter* sp. cells in experiments with ¹⁴C-labeled Na-humate. Decomposition of humic acid is believed to occur more rapidly by mixed than by single cultures of microorganisms (Andreyuk et al., 1973). However, Kononova (1970) seems to dispute the possibility of absorption of humic acid by microorganisms, since cleavage of humic acids in most of the work cited has not been established. McLoughlin and Kuster (1972) also report conflicting evidence by indicating that humic substances have no effect on growth and respiration of the yeast *Candida utilis*. Studies on the effect of fulvic acids on the growth of an ectomycorrhizal fungus, *Pisolithus tinctorius*, as conducted by Tan and Nopamornbodi (1979), yield results showing evidence of definite absorption of fulvic acids. Moderate amounts of fulvic acids (640 ppm) seem to have stimulated the growth and dry weight content of the ectomycorrhiza, cultivated at pH 7.0 and 4.0 (Figure 10.4). The mycelia of colonies developed at pH 7.0 are substantially darker in color than the cultures grown at pH 4.0. The possibility of fulvic acid being absorbed by the fungus, as indicated by the distinct discoloration of its mycelia, is supported also by the fact that fulvic acid has been found to be composed of polysaccharides (Tan and Clark, 1968; Clark and Tan, 1969). Fungal cells are known to contain large amounts of polysaccharides; hence, it is likely that fulvic acid is an important food source by these organisms.

10.5.5.2 Aquatic Organisms

In more recent reports, humic substances are also considered important for organisms living in aquatic environments, although the effect is almost the same as that discussed above for soil organisms. Some believe that humic substances cannot serve as a heterotrophic food source, but many agree that

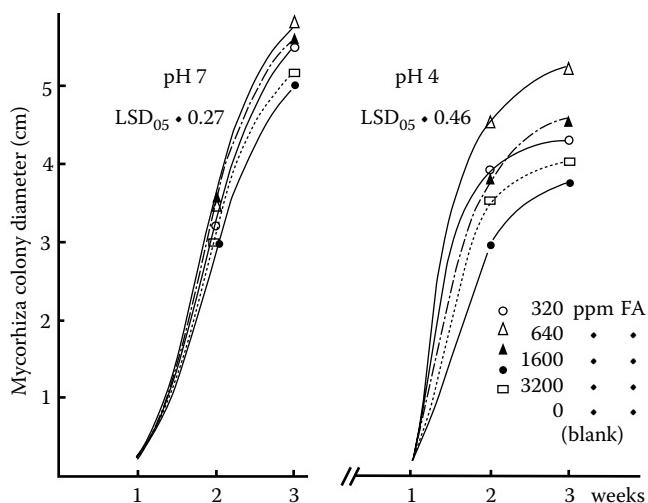


Figure 10.4 Effect of fulvic acid (pH 7.0 and 4.0) on colony growth of the ectomycorrhizal fungus, *Pisolithus tinctorius*. (From Tan, K. H., and V. Nopamornbodi, *Soil Biol. Biochem.*, 11, 651, 1979.)

aquatic microorganisms are capable of using their enzymes to attack the free carboxyl end of humic substances (Harvey and Boran, 1985). Others claim that the growth of these microorganisms *in vitro* increases in the presence of humic substances at concentrations of 30 mg/L in the growth medium (Visser, 1986). This is supported by an earlier report evidencing increased bacterial biomass and activities due to the presence of aquatic humic matter (Stewart and Wetzel, 1982). It is assumed that the humic substances are used as an N source (Claus et al., 1997), or are utilized by a cometabolism mechanism by the aquatic microflora (De Haan, 1977). Cometabolism is a process by which the humic substance is taken up by the organism but is not used as food or a source of energy. Apparently it is useful for cell growth and in assisting decomposition of substances by the cell. Other modes of action of humic substances are their auxin-like action, decoupling of oxidative phosphorylation, and their effect on regulating membrane permeability (Flaig, 1970; Chaminade and Blanchet, 1953). A more recent finding on membrane permeability is given below to illustrate the uncertainties underlining the issue. Although not much is known for sure in this aspect, the little information available, mostly coming from histological research, reveals the likelihood of increased permeability of membranes of aquatic organisms by humic substances. Studies by Tham et al. (1996) on the population of macroinvertebrates in bog streams in Germany indicate that some taxa of

the Diptera and Crustacea have been decreasing in density with increasing humic matter content in the streams. The possibility is raised that some of the humic matter has penetrated the membranes of the organisms, causing harmful subcellular reactions, an assumption deduced from changes noted in the osmotic reactions of a *Trichoptera* sp. Similar subcellular reactions have also been quoted by the authors on gills of brown trout. Hartung and Allread (1994) seem to corroborate the damaging effect by noting an algicidal effect of humic acid in their research. Results of their pond tests show that peat humic acid can provide algae control at low concentration, although the data are hardly statistically significant. The authors believe that the algicidal reaction is induced by solar radiation. However, no damage by the humic acid is noticed in other forms of aquatic life, e.g., fish, frogs, turtles, snails, daphnids, and worms. The fact that many types of fish, alligators, and other aquatic organisms are thriving well in black water rivers of the world and in the humic water of the Okefenokee Swamps of South Georgia in the United States is intriguing, and more information has to be collected to definitely confirm the harmful effect of humic acid on some aquatic life.

Another aspect of great environmental significance is the complex reaction between live microorganisms and humic matter and soil clays. Because of their sheer number in soils and aquatic environments, microorganisms are bound to form complexes with clay minerals and humic substances. The author believes that these microorganisms play a dual role in humic acid chemistry, as examined below. Numerically, the population of microorganisms is the largest among all soil organisms, and no doubt their cells will participate as important components in the self-assemblage process forming humic assemblies. In addition, microbial cells are believed to also interact with humic matter in much the same way as the interaction between clay and humic acids or humic acid and enzymes as discussed earlier. The difference is only that microorganisms, such as bacteria, are too large to be classified as soil colloids, but nevertheless they behave as colloids. The colloidal behavior is manifested in repulsion between the negatively charged bacterial cells, causing them to disperse in aqueous solutions. Formation of such suspension is a characteristic known to be exhibited only by colloids. Another contrasting difference is that bacteria are living cells that possess the capability to grow, exhibit metabolism, and move independently. In contrast, however, the larger (higher) soil flora and fauna will react differently with humic matter or clay. They are generally too large and well insulated to be affected by the charge characteristics of the humic and mineral fractions. In analogy to the preservation of organic matter in soils as a result of

complex formation with clays, the interaction of microbial cells with humic matter or clays also ensures the survival and accumulation of certain groups of microorganisms, their enzymes, and metabolic products. The adsorption of protein by clays and humic matter and their subsequent protection from decomposition has been known for many years and thoroughly discussed above. Hence, it is reasonable to expect that a microbe–humic complex provides some protection for the organism from physico-chemical attack by other microorganisms. Although the discussion above underscores only the interactions between humic matter and soil organisms, it does not infer that complex reactions between clay and soil organisms are second only in importance. It plays an equally significant role in affecting microbial life in the soil ecosystem, and for more details on interaction reactions between clays and microorganisms, their kinetics, and mechanisms, reference is made to Tan (1998).

10.6 Importance in Plant Growth

The effect of soil organic matter on plant growth has been known for some time; however, only recently has this effect been credited to humic compounds. Humic matter enters into complex reactions with a variety of organic and inorganic components of soils and will influence plant growth and crop production indirectly and directly. Indirectly, it is known to improve soil fertility by modifying physical, chemical, and biological conditions in soils. These effects have been discussed in some detail in the previous sections. Therefore, this section will discuss only the direct effect of humic acids. With the increased knowledge in humic acid chemistry, starting a decade ago, increasing amounts of information have been accumulated, testifying to the profound influence of humic matter on plants. The reports are formidable not only because of the huge amounts of data but also because of the variety of effects allegedly exerted by humic substances. Almost any aspect of plant life seems to be affected in one way or the other, from plant nutrition and processes in plant physiology to plant growth and crop production.

10.6.1 Effect on Plant Nutrition

The opinion exists that humic acid can serve as plant food, and much of the older literature attests that small amounts of organic substances can stimulate plant growth.

10.6.1.1 Humic Matter as a Food Source

This concept finds its origin perhaps from the experiments of John Woodward, who reported late in the seventeenth century that plants were growing better in muddy water than in rain or river water (Tan, 2000; Brady, 1990). This led his critics to conclude that humus was taken up by the plants, a theory revived in the beginning of the twentieth century by results of experiments conducted by people using compost and peat extracts for growing plants. Added in small amounts to nutrient solutions, the organic extracts were believed to have a stimulating effect on the growth of duckweed (*Lemna minor* or *Lemna major*) plants (Mockeridge, 1920; Visser, 1986). These results were disputed by Clark (1924), who indicated that the added organic substances affected the growth of microorganisms capable of producing growth-stimulating substances that caused the better growth of the duckweed. With the increased knowledge on humic acids, starting a few decades ago, more information has accumulated on the direct effect of humic matter on plant growth. Inconclusive reports suggesting at first that small degradation products of humic acid can be taken up by plants (Flaig, 1975) were later defended by claims that penetration of root membranes by humic molecules is made possible by a depolymerization or depolycondensation process (Dell'Agnola and Nardi, 1986; Burns, 1986). Roots are believed to cause a considerable reduction in pH of the soil rhizosphere by releasing a variety of organic acids. These root exudates, e.g., oxalic, fumaric, malic, citric, and succinic acids, act as depolycondensation agents on humic acids. Dell'Agnola and Nardi (1986) are of the opinion that the organic acids cause the transformation of high molecular weight humic acid into humic fractions with low molecular weights (m.w. <5000). However, it is noted that this depolycondensation process is reversible and that the transformation is strictly dependent on the presence of organic acids and low pH values (pH 2.5). From a more recent investigation, it is asserted that root exudates from corn plants (*Zea mays* L.) are capable of converting insoluble humic acids into soluble forms (Nardi et al., 1997). Although Nardi and coworkers did not elaborate, the present author believes that the increased solubility of humic acid contributed to its rapid depolymerization. In the acidic environment, the soluble humic matter is an easy target for hydrolysis in breaking down the humic molecule. The contention that small humic fractions can serve as food sources for microorganisms seems to be supported by earlier results. In their experiments growing an ectomycorrhizal fungus (*P. tinctorius*) with fulvic acid, Tan and Nopamornbodi (1979) observed discoloration of fungal mycelia.

Mycelia turning yellow to dark brown demonstrates absorption of the fulvic compound. Known to be the smallest humic fraction, fulvic acid is composed mostly of polysaccharides (Tan and Clark, 1968; Clark and Tan, 1969), a valuable food source for microorganisms. The ecological significance of this result is enormous. Fulvic acid is expected to be present in soils in amounts larger than sugars. Therefore, it is more readily available than sugars. Microorganisms that can use fulvic acid as food or as an energy source are less susceptible to fierce competition than those that can only use sugars.

More and more scientists today seem to accept the idea that these small humic fractions can be taken up by plants. Higher plants are, in this respect, compared by some as facultative heterotrophic organisms capable of consuming small fragments of humic acid (Popov and Chertov, 1997). The lower molecular weight humic fractions are believed to be active in cell differentiation and in stimulation of enzyme activities.

10.6.1.2 Humic N as Plant Nutrient

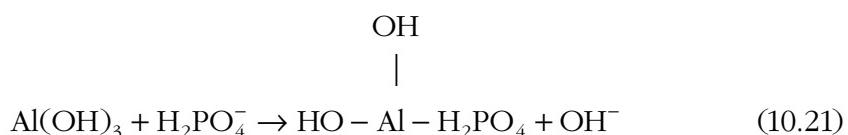
Humic substances are known to contain appreciable amounts of N. The N content of humic matter is reported to be in the range of 2% to 5%, with humic acids generally exhibiting N contents in the higher range and fulvic acids in the lower range (Tan, 1998; Cranwell and Hayworth, 1975; Schnitzer and Khan, 1972). Most of this N is assumed to be present as amino acid N, acid-insoluble N, and hydrolyzable unknown N (HUN). Lower amounts are available in the form of NH₃-N and amino sugars. Although Stevenson (1994) assumes that one-half of the total N in humic matter can be accounted for as amino acid, Rossell et al. (1978) indicate that the bulk of N is in the acid-insoluble N fraction. In view of the above, it is expected that most of the N is relatively stable and available only with difficulty for growing plants. However, most scientists agree that after decomposition of the humic molecule, the fixed N can be released and made available to growing plants. Since in laboratory analyses, acid hydrolysis is the process by which amino acid N is liberated, it can be expected that a similar hydrolysis reaction is playing a major role in natural conditions. Although one theory assumes that N is released as N₂ gas, the more common concept is that it is released in the NH₃ form with the assistance of heterotrophic microorganisms. Production of N₂ gas would have completed the N cycle; however, at this stage, this does not conform to the N cycle. On the other hand, the production of NH₃ concurs with the flow of the biochemical reactions by allowing this stage to be followed by the nitrification process in continuation of the N cycle (see Figure 10.3). The possibility of such a decomposition of humic N and its subsequent

utilization by microorganisms have recently attracted some investigations by a team of German and U.S. scientists, using aquatic humic acid collected from the St. Mary's River in Georgia, United States. The results of their laboratory experiments seem to indicate that the autochthonous microflora of the river water were capable of utilizing humic N as a source of N (Claus et al., 1997), as mentioned briefly in an earlier section above on the effect of humic matter on soil organisms.

In addition to being a food source, humic acid has also been reported to increase N uptake by plants and to enhance plants' capability of utilizing N in nitrogen-deficient soils. The increased uptake is believed to have, in turn, a stimulating effect on the synthesis of N compounds in plants (Chaminade, 1958, 1966).

10.6.1.3 Humic Phosphate Nutrition

Humic matter is not considered to carry P in its molecule in adequate amounts for plant growth, unless one is arguing about biologically fixed phosphates, which will be discussed at the end of this section. However, it is known that humic matter can affect the solubility of insoluble P compounds in soils. Its chelation capacity is a major force for decomposing rock phosphates and other insoluble forms of phosphates in the soil, e.g., AlPO_4 and FePO_4 . The strong affinity of Al and Fe ions in aqueous solutions for phosphate ions rapidly converts soluble P fertilizers into insoluble AlPO_4 and FePO_4 by a process called phosphate fixation. This process is especially important in acid soils where the Al and Fe concentrations are generally very high. The presence of humic matter is believed to increase the solubility of the metal phosphates by reducing the activity of Fe and Al ions by complexation (Del Re et al., 1978). Sinha (1971, 1972) also found that fulvic acid increased the solubility of insoluble phosphates because of complexation of the metal ions by fulvic acid. Such an interaction results in a release of part of the phosphate as free ions, which can be illustrated by the following reactions:



This high affinity of humic substances for Al, Fe, and Ca is a major reason why they compete for these elements with phosphate ions. Lobartini et al. (1994) have confirmed the enhanced dissolution of the phosphate mineral due to interaction with humic acid, whereas in a more recent investigation Lobartini et al. (1998) underscore the role of humic acid as a powerful chelator of Al and Fe. The dissolution of AlPO_4 and FePO_4 by humic acids yields mostly free orthophosphate ions, as detected by their ^{31}P -NMR (nuclear magnetic resonance) analyses. Corn plants grown by Lobartini and coworkers in hydroponics, with AlPO_4 and FePO_4 as the sole source of P, exhibit with the addition of humic acids a similar, if not better, performance than those grown with KH_2PO_4 as the P source. Indications are also present that some of the phosphate is possibly converted into a fulvo–metal–P complex compound. The latter, called coadsorption or metal bridging, has been reported earlier by Weir and Soper (1963), who believe that humo–Fe complexes, in addition to humic matter only, can also attract P yielding humo–Fe–P complexes. The interaction is a metal-bridging process and can be illustrated as follows:



Such an interaction is considered a form of biological fixation of phosphates. Another form of biological fixation of phosphates is the immobilization of phosphates into the cellular material of microorganisms. Although biologically fixed phosphate is biologically stable, it is more soluble than the fixed Al- and Fe-phosphates. Because organic matter is always subject to decomposition and mineralization, the phosphates fixed biologically can be released more easily than their inorganically fixed counterparts. Many of the organically bound phosphates also exist in soluble forms; hence they play a vital role in replenishing inorganic P in the soil solution. For a more detailed discussion on the biological fixation of phosphates, their products, and decomposition, reference is made to Tan (1998) and Stevenson (1994).

10.6.1.4 Humic Macronutrient and Micronutrient Relations

It should be emphasized perhaps that humic matter cannot be considered a fertilizer since it does not contain any of the nutrients as fertilizers do. The plant nutrients are carried only by virtue of its large cation exchange and chelation capacity. In many acid soils, the exchange and chelation sites may be saturated with H^+ ions, and only when these soils are fertilized can these sites be saturated with macronutrients and micronutrients. Hence the exchange sites

serve as storage for large quantities of available nutrients for plant and microbial growth. Plant roots can obtain the adsorbed cations by cation exchange, using H⁺ ions, produced as by-products of root respiration, as exchange materials. This exchange phenomenon between humic matter and plant roots is also important for the salt balance of the soil ecosystem. High salt concentrations, usually toxic to growing plants, can be alleviated by adsorption by humic acid. It is also believed that this exchange facilitated by humic acid allows plants to grow within a wider pH range (Badura, 1965).

Although by rules of soil chemistry, chelated metals are more strongly bonded than those on exchange sites, depending on pH, the chelated metals can also be made available to plants by exchange. From their investigations, Lindsay (1974) and Lindsay and Norvell (1969) note that at pH 7.0, Ca²⁺ ions can release Zn and Fe from Zn-EDTA and Fe-EDTA complexes. At low pH, Fe³⁺ ions can displace Zn²⁺ from Zn-EDTA complexes. Since most of the humo-metal chelates remain soluble, they will move with the flow of soil water, hence provide the carrier mechanism for transport to the soil-root interface. The metal chelation properties of humic matter find application today in the fertilizers industry in Europe, which will be discussed in more detail in the section on industrial importance in Chapter 11.

10.6.2 Effect on Plant Physiology

A number of reports indicate that humic matter has, in general, a stimulating effect on plant respiration and photosynthesis.

10.6.2.1 Plant Respiration

A significant increase in respiration of barley plants (*Hordeum sativum*) grown in nutrient solutions due to the application of humic substances was reported by Khristeva (1953). The consumption of O₂ by these plants is noticed to double within a 60-min period. According to Sladky and Tichy (1959), spraying begonia plants (*Begonia semperflorens*) with humic acid also affected plant respiration, as displayed by a release of 17% to 30% more CO₂. Other plants, whose respiration processes have been observed to be stimulated one way or the other by humic substances, are tomatoes (*Solanum lycopersicum*), corn (*Zea mays*), wheat (*Triticum vulgare*), and gourd (*Cucurbita maxima*) (Sladky, 1959; Šmídová, 1962). These increases in endogenous respiration are noticed not to be accompanied by concurrent increases in rate of glycolysis, the breakdown of carbohydrates. While, on the one hand, respiration processes are apparently increased,

and, on the other hand, humic substances reduce glycolysis, the two processes raise concerns about producing an imbalance in metabolite concentrations in plant tissue (Prát, 1970; Visser, 1986).

10.6.2.2 Photosynthesis

The effect of humic substances on photosynthesis has also attracted some attention in soil science. Sladky (1965) claims that sugar beets (*Beta vulgaris*) exhibit a 22% increase in adsorption of CO₂ as a result of application of humic acid sprays at 300 mg/L. Others testify that humic matter increases the chlorophyll content of several plants, e.g., water flaxseed (*Spirodela polyrhiza*), sugar beet, and tomatoes (Visser, 1986). Whether an increase in chlorophyll brings about an increase in photosynthesis is still a matter of conjecture. The argument can be presented that the stimulating effect on chlorophyll content of green plants can be used to control chlorosis and hence improve photosynthesis.

10.6.2.3 Metabolite Content

Humic acid is reported to affect the carbohydrate content in plants. The free sugar content in plant tissue is found to increase, in general, by humic acid application. This effect of humic acid is believed to be different for the different plant parts. Lower increases have been observed in stems and leaves than in roots, which Cinćerová (1962) attributes to the substantial increases in growth of the above-ground vegetative part as a result of humic acid application. The accumulation of sugars may affect other important physiological functions of the plants. Increasing the cellular sugar content generally increases the osmotic pressure inside plant cells. Flaig and Saalbach (1959) believe that this high osmotic pressure is the reason for the resistance to wilting of plants grown in the presence of humic matter. Some reports also indicate a beneficial effect of humic acid on the alkaloid content of plants. Limited investigations with tobacco (*Nicotiana tabacum*) and several medicinal plants show a stimulating effect of alkaloid contents in the leaves due to humic acid application (Aitken et al., 1964; Tolpa, 1976).

10.6.2.4 Membrane and Protoplasm

The increased uptake of nutrients by plant roots due to humic acid applications has led many authors to believe that humic matter can increase

membrane permeability (Khristeva, 1953; Chaminade, 1956; Lee and Bartlett, 1976). It is believed that by affecting the electrogenic proton pump, the permeability of the plasmalemma is increased (Zientara, 1983). Increased transport of glucose has been observed across the membranes of onion (*Allium cepa*), sugar beets, and sunflower (*Helianthus annuus*) cells due to humic acid applications (Blagowestschenski and Prosorowskaja, 1935). However, used at high concentrations (1500 mg/L), humic acid can cause cell membrane damage (Vaughan and MacDonald, 1976) and increased hydration of protoplasma colloids. Otherwise, humic substances used at low concentrations (10 mg/L) are noticed to increase plasma flow in cell tissues, indicating a reduction of cell protoplasm viscosity. The results of a more recent investigation provide additional support for the effect of humic acid on increasing cellular plant membrane permeability. Irintoto and Tan (1993) noticed callus cultures of slash pine (*Pinus elliottii* Engelm) in a Brown and Lawrence medium to increase in weight in the presence of humic acids over the control (no humic acid). The elemental composition of single callus cells, determined by energy dispersive analysis by x-rays (EDAX), also indicates a substantial increase over the control in P, K, Ca, and S contents due to treatments with 160 µg HA/mL (Figure 10.5). The authors above attributed this to the effect of humic acid in increasing the permeability of callus membranes.

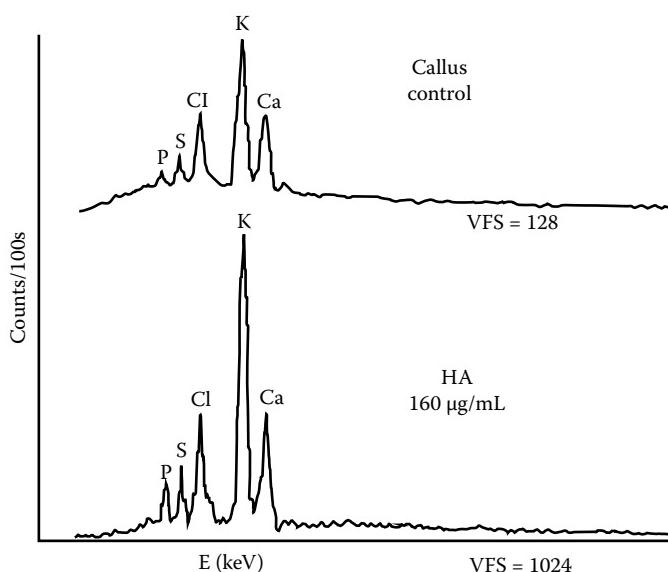


Figure 10.5 Elemental composition of single callus cells of slash pine (*Pinus elliottii* Engelm) as determined by energy dispersive analysis by x-rays (EDAX).

The increased uptake and, consequently, greater accumulation of nutrients in callus cells are then the reasons for callus weight increases due to humic acids.

10.6.2.5 Plant Growth and Crop Yield

Many of the investigations with humic acids are limited to studying seed germination, shoot growth, and elongation of very young seedlings or elongation of excised roots *in vitro* (Tan and Nopamornbodi, 1979a; Vaughan, 1974; Poapst and Schnitzer, 1971). The results invariably show an effect similar to a hormonal growth effect. On the other hand, work on nutrient uptake by Guminski and coworkers in Eastern Europe (Guminski et al., 1977; Guminski, 1957) and by Dormaar (1975) in Canada reveals a physiological influence of humic acids on plant growth. From studies on the growth and nutrient uptake of corn plants, Tan and Nopamornbodi (1979a) came to the conclusion that humic acid affected plant growth through a combination of the aforementioned processes. Moderate amounts of humic acids were generally beneficial for root and shoot growth of the corn plants (Figure 10.6). At the same time, a significant increase in N content of shoots of the corn seedling was obtained. Dry matter production also appeared to be stimulated. Data presented by Visser (1986) seem to support the idea that humic acid can beneficially affect the N contents of plants.

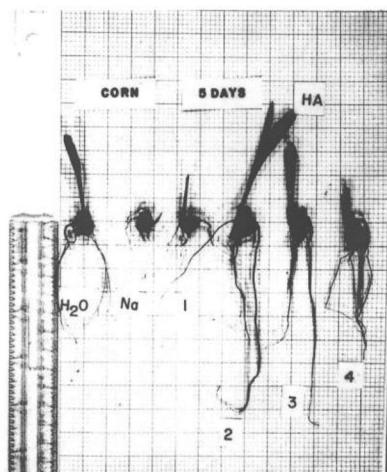


Figure 10.6 Effect of humic acid on germination and growth of 5-day corn (*Zea mays L.*) seedlings grown in a Hoagland solution to which the following were added: (H₂O) 0 ppm HA, (Na) blank + 0.66 meq NaOH, (1) 320 ppm HA, (2) 600 ppm HA, (3) 1600 ppm HA, or (4) 3200 ppm HA.

In the presence of humic acids, uptake of N by plants is increased significantly, and plants are noted to be able to take up amounts of N at concentrations normally considered toxic to their growth. Flaig (1953, 1956) and Kononova (1956) indicate that roots are more affected by humic acids than the above-ground vegetative part. Others believe that the effect of humic acids depends on plant species. On the basis of the effectiveness of humic acids, the following distinction of plants into four groups has been suggested (Khristeva, 1953; Khristeva and Manoilova, 1950):

1. Carbohydrate-rich plants, e.g., potato, sugar beets, carrot, and tomato, showing a highly positive response to the application of humic acid
2. Cereals, e.g., barley, oats, wheat, rice, and corn, showing a moderate reaction to application of humic acid
3. Protein-rich plants, e.g., green beans, peas, and lentils, showing a minimal reaction to humic acid applications
4. Oil-producing plants, e.g., castor-oil plants, cotton, linseed, and sunflower, which are not affected or will be negatively affected by humic acid

Whether such a division can be upheld is a matter of conjecture, although the results of a follow-up investigation reveal no effect of humic acid on oil-producing plants, whereas only a small effect is noted in legumes (Chaminade, 1958, 1966).

Today, humic acids have also been produced commercially by various companies in the United States and other countries for use as soil amendments to enhance crop yields. Websites, e.g., www.yahoo.com, www.google.com, www.mnsb.com, etc., are loaded with a great many companies offer on the Internet all kinds of humic acid products, called *humates*, with fantastic claims in crop yields by the use of these humates. The issue of these so-called commercial humates is examined in Chapter 11. Here an example is presented involving the most recent report on an allegedly beneficial effect of humic substances on potato yields from field experiments conducted by Selim et al. (2010). Selim and coworkers reported that potato plants grown on sandy soils of the Agricultural Research Station of the National Research Center, Giza, Egypt, increase their tuber yield from 41.4 Mg/ha in the control to 47.77 Mg/ha in potato plants treated by fertigation with humic acid solutions. According to their calculations, the difference in yield of 6.4 tons/ha is statistically highly significant.

Chapter 11

Environmental and Industrial Importance of Humic Matter

11.1 Importance in the Environment

Because of its unique electrochemical and ion exchange behavior, humic matter plays a significant role in the environment. Its function in soils and the biochemical cycles are so vital that many, if not all, biochemical activities will come to a standstill in the absence of humic matter. Degradation of soils starts with the destruction of humic matter, bringing with it all kinds of environmental disasters.

In preceding chapters, humic matter has been discussed as a participant in the carbon and nitrogen cycles, two important cycles for maintaining a healthy environment in the soil's ecosystem. Fixation of organic carbon and proteinaceous compounds in the form of humic matter functions as part of the cycles, enriching soils with much-needed organic matter and nitrogen for survival of soil organisms. By later decomposition of humic matter, the fixed carbon and nitrogen are released again, enabling continuation of the respective cycles. Without the synthesis of humic matter, most of the carbon will be lost rapidly in the atmosphere in the form of CO_2 gas, leaving the soil void of organic matter. The ensuing disastrous effects of soil degradation can be prevented through management practices aimed at the accumulation and maintenance of sufficient amounts of organic matter in soils. For more details, reference is made to Tan (2000).

11.1.1 Preservation of Soil Organic Matter

It is now well known that soil organic matter is perhaps the most important soil component that has attracted a lot of attention since the early days of agriculture. Regardless of opinions to the contrary, it is difficult to dispute the fact that the growth and yield of crops are always better in soils rich in organic matter. The accumulation and preservation of organic matter in soils is often called *organic carbon sequestration* and generally a process that is biological in nature. As such it is an essential part of the carbon cycle, as examined in some detail in Chapter 10. Formation of peat and establishing the growth of forest and/or other types of vegetation naturally or by cultivation are examples of biological sequestration of carbon. With the rapid industrial and agricultural revolutions in the past century, huge amounts of CO₂ gas are produced and its emission is causing serious problems in polluting the earth's atmosphere, as noted lately. The capture and storage of the CO₂ and other carbon gases from the so-called flue gases by artificial techniques are known today as *geoengineering*. A great deal of attention and concerns have been expressed by the scientific community, government institutions, and especially the politicians that wholesale emissions of CO₂ into the air and other forms of produced industrial carbon gases are the cause of global warming and/or other climatic changes on Earth (Tan, 2000). However, this book is not the appropriate place for discussing the general aspects of organic carbon preservation and storage by biological processes, and issues of geoengineering. The intention is to examine only the very specific part of the biological process related to humic substances affecting the capture and storage of organic carbon. Two specific examples are chosen to illustrate in the following two subsections the accumulation and storage of organic carbon because of the exceptional types of humic acid formed unique to the soils in question. Many types of other examples are also available, but due to lack of uniqueness, it is neither in the interest of this book nor that of its audience to examine them all.

11.1.1.1 Ca Humates in Mollisols

One example of soils where organic carbon is captured and stored in large amounts in the form of organic matter in general and in humic acids in particular is the soils of the Great Plains in the United States. These soils are classified by U.S. Soil Taxonomy as *mollisols* (Soil Survey Staff, 1990) but are known in Russia more under the name of *chernozems*. Mollisols

or chernozems are the most fertile soils in the world. Their high contents of organic matter are one of the main reasons for their capability to support huge productions of corn, wheat, and soybean. Because of this, the regions with mollisols in the United States were given the name *corn belt* in the past, whereas in Russia, the regions occupied by chernozems are dubbed as the *wheat belt* of Europe. Large areas of mollisols are known today to also occur in Canada, Argentina, the loessic and steppe areas of the Gobi regions of China, and part of the Sahara deserts of Africa. The chernozemic soil profile used by Dokuchaiev as the classical model of a soil profile has contributed greatly in advancing the classical *pedological concept* in soil science. The soils are generally characterized by thick, black A horizons, containing high amounts of soil organic matter. Under natural conditions, a C_{org} content of 6% has often been reported in the past, which translates into 10% to 12% soil organic matter. Especially tall grass vegetation and semihumid climatic conditions, ensuring an annual turnover of abundant amounts of organic matter and a small degree of leaching, are important factors for the accumulation of organic matter and the formation of humic substances. The humic substances formed are saturated with Ca²⁺ and/or Mg²⁺ ions. These ions are derived from the decomposition of calcareous and loessic parent materials and because of the limited leaching process are accumulating in the soil solution. The Ca humates can be precipitated as such, or are immobilized after interaction with smectites or montmorillonites through Ca-bridging mechanisms (see Chapter 9 and Figure 9.6). In the form of humo-Ca-smectite complexes, preservation of organic matter (humic acids) is ensured and maintained.

11.1.1.2 Al Humates in Andosols

Another outstanding example of soils rich in organic matter is the *andosols* (Arnalds, 2013; Tan, 1984, 2008). These soils are derived from volcanic ash and classified by U.S. Soil Taxonomy as *andisols* (Soil Survey Staff, 1990). They were known in the past also under various names, e.g., *humic-allophane* soils (Kanno, 1962), referring to humic acids and allophane as the most characteristic components in the soils. These soils are black because of their high organic matter contents. In contrast to mollisols, andosols are acid soils rich in Al and Fe and amorphous clay minerals, e.g., allophane and imogolite. The general consensus is that these clay minerals together with amorphous silica play a major role in the accumulation of humus, producing the intense black A horizon and the development of physical and chemical

properties unique to andosols. Exposed groups of Al and Si on the surfaces of the amorphous clay minerals are capable of interacting with humic acids, forming humo-Al-allophane or humo-Si-imogolite complexes or chelates (see Figure 9.6). These chelates increase the resistance of humic acids against chemical decomposition and microbial attack. The process is considered one reason for the high organic matter content in andosols. Because of their favorable physico-chemical properties created by the high humic acid contents, these soils are favored worldwide for a variety of agricultural operations and in particular for growing horticultural crops (Tan, 2008). In the high mountain soils of the Andes in Peru, andosols are the home for Irish potatoes. In Southeast Asia, especially in Indonesia, the andosols are often considered, next to climatic requirements, the main factor for successful horticultural operations. Due to the soils' location in cool tropical mountain climates, cabbages, tomatoes, carrots, Irish potatoes, green onions, and other temperate region vegetables and also cut flowers are grown for local farmers' markets, restaurants, and supermarkets, or commercially for export to Singapore and other neighboring Southeast Asian countries. The best tea and coffee plantations are found on andosols. The quality of the so-called highland or mountain tea is considered superior over that of lowland tea that is usually grown at lower elevations on different kinds of soils (Tan, 2008).

11.1.2 Soil Nitrogen and Humic Matter Content

The most striking effect of deforestation is the decrease of soil organic matter content, which has an impact on nutrient cycling. Then the process of degradation of soils starts to increase, although it can be slowed down somewhat by the use of proper cultivation and management practices. Such a cultivation system has been known in the tropics for decades under the name *plantation agriculture*, and recently a variation of this method has been introduced in the temperate regions under the name *agroforestry* (Tan, 2000).

A natural rainforest ecosystem has a huge standing biomass and large amounts of nutrients are contained in the plant biomass. These nutrients are usually released upon decomposition of the litter. A dense vegetative cover will add substantially to the organic matter content in soils, which is the main, if not the sole, source of soil nitrogen. It is now an established fact that soil nitrogen content increases with increased organic matter content. A positive correlation exists between organic carbon and total nitrogen

contents, which usually takes the form of a linear regression as follows (Tan and Troth, 1981):

$$N = a + bC \quad (11.1)$$

where N = % total soil N, C = % soil C_{org} , a = intercept, and b = regression coefficient.

The equation above indicates that total nitrogen content increases linearly as organic carbon increases in the soils. As discussed in earlier sections, humic acids contain 4% to 5% N, and hence are expected to be important contributors of soil N. Therefore, destruction of soil organic matter and especially the humus content may have serious implications for soil nitrogen contents.

11.1.3 Mobilization and Immobilization of Elements

Reduction and oxidation reactions play a significant role in the mobilization and immobilization of soil elements, contributing to soil formation, soil fertility, and decomposition of xenobiotics. In a preceding section, it was discussed that humic matter was capable of donating-accepting electrons, and in doing so, can change the redox status of soil elements, many of which are essential as plant nutrients. The capacity of humic matter in transferring metal ions from their oxidation state into the reduced state assists in making them more soluble, hence more available for plant uptake. The mobilization of Al, Fe, and Mn due to changing their redox status is also an important process in soil genesis. Movement of Al, Fe, and Mn in the form of humochelates from the A to B horizons allows for horizon differentiation in the pedon. The transportation and subsequent deposition as nodules and ores in wetlands and marine strata are additional examples. All of these are vital environmental processes enabling the formation of spodosols, paddy soils, and valuable mineral deposits.

This redox behavior of humic matter is also believed capable of affecting *photochemical* reactions in the soil environment, which contributes to a photochemical breakdown of a number of xenobiotics. This is a *detoxification* reaction, which will be discussed in more detail in the following section.

Other important reactions affecting mobilization and immobilization of cations are complex reaction and chelation. The dissolution of many of the metals, e.g., Al and Fe, is usually increased by a complex reaction and/or chelation with humic substances. At relatively low “loads” of cations,

leaving the complexing sites still unsaturated, the humo–metal complexes are soluble. These complexes or chelates retain their negative charges, although the latter decrease proportionally by increased complexation of cations, until at saturation all negative charges of the humic molecule become zero. At this point, the humo–metal complexes and/or chelates are precipitated out of the soil solution; in other words, the metals are immobilized.

11.1.3.1 Significance in Soil Genesis

The processes of mobilization and immobilization of metal ions through formation of chelates and complex compounds have an important bearing on soil genesis and soil fertility. They are considered of utmost importance in the development of specific horizons characterizing the pedon. Current concepts on formation of *spodic horizons* in spodosols are based on the concept of mobilization and immobilization of Al and Fe. As discussed above, the stability and mobility of Al and/or Fe chelates depend on the saturation of the reaction sites with the metal ions. If low amounts of Al and/or Fe are available for complex formation, complexes or chelates will be formed in the A horizon with low metal/HA ratios. In this case, the amount of Al and/or Fe chelated is insufficient to cause immobilization of the metal–HA compound, which may then move down the pedon with the percolating water (De Coninck, 1980). During the downward migration, the metal chelates may pick up more of the polyvalent cations, resulting in a progressive decrease of the negative charge. The presence of a higher metal ion concentration in the subsoil, or an acidity different from that in the A horizon, may eventually neutralize the remaining charges. The consequent precipitation of the metal–HA chelate gives rise to the development of a spodic horizon.

On the other hand, when sufficient amounts of Al and/or Fe, or other metal ions can be released by mineral weathering so that metal–HA chelates can be formed with high metal/HA ratios, the chelates are then immediately immobilized in the A horizon before they can migrate down the pedon. The formation of mollic epipedons is an example of the immobilization of Ca–HA chelates attributed to saturation of the reaction sites of the humic molecule with Ca ions. Another example is the formation of andic horizons, characterizing andosols, due to immobilization of Al–HA and allophane–Al–HA complexes (Wada and Aomine, 1973).

11.1.3.2 Significance in Micronutrient Nutrition

The role of mobilization and immobilization of metal chelates in soil fertility can be illustrated as follows. The metal ions—by themselves normally insoluble at the prevailing soil pH—can remain soluble because of chelation and/or complex formation and may then move as chelates by diffusion and mass flow to plant roots. Many of the chelated cations are essentially micronutrients, and the chelates are considered to provide the carrier mechanism by which depleted micronutrients in the root zone are replenished (Lindsay, 1974). The chelated nutrients can be made available to plant roots by exchange. This exchange is quite different from the classical or normal cation exchange as a result of coulombic attraction in a double layer. As discussed in an earlier section, the chelated metal cannot be replaced by, for instance K^+ or Na^+ , which are not capable of occupying the position of a Cu^{2+} ion or any other transition metal in a chelate. A chelated metal can only be exchanged by another transition metal. Depending on pH, Lindsay (1974) indicates that Fe^{2+} in chelate form can be replaced with relative ease by Zn^{2+} and vice versa.

11.1.4 Biological Detoxification

The exchange and chelation capacity of humic matter provide for a huge buffer capacity in soils and the environment. Over the years, increasing amounts of a variety of agrochemicals find their way into the soils, including large amounts of pesticides, agricultural and industrial wastes, and their residues, fouling up the earth's soil and hydro-ecosystems (Tan, 2008).

11.1.4.1 Role in Soil Hygiene as Eco-Friendly Detoxifiers

The substances from industry and agricultural operations, contaminating the soils, are called today *xenobiotics*. Many of the pesticides and their residues are toxic to soil biota, animals, and humans, and only very few are not harmful to the fauna and flora in our environment. Fortunately most of the harmful materials are adsorbed or chelated by humic matter, and their activities either reduced or canceled. As such the humic acids are cleaning the dirty conditions in the soil by acting as *ecologically friendly* detoxifiers, an issue referred to by some soil scientists as closely related to enhancing "soil hygiene."

As discussed earlier, the heavy metals Al, Fe, Cu, Zn, Mn, Co, Hg, and Cd originating from sewage sludge disposed of in soils are deactivated due to chelation by humic acids. This role of humic matter is also noted to be important for detoxification of radioactive elements in relation to nuclear waste disposal. The impact of humic matter is realized in this case as a confinement or as an enhanced migration of the radioactive elements in the environment (Moulin et al., 1996). However, other scientists are of the opinion that humic substances do indeed have a reducing effect on toxicity and bioaccumulation of heavy metals and organic chemicals. The results of biotesting experiments by Perminova et al. (1997) on the photosynthetic activity of green algae (*Chlorella vulgaris*) show a detoxification effect by humic acids on Cd, Cu, and Pb. A variation in mode of detoxification has also been noticed in that marine humic substances have displayed weaker effects than their soil counterparts. Differences in structural composition are assumed to be the reason for this, since marine humic matter is considered to be more *aliphatic* in nature, which allows for a smaller chelation capacity than the highly *aromatic* humic substances in soils and peats. Hence, concerns have been raised by the authors that marine environments can be more easily affected by toxic contamination with heavy metals than the soil ecosystem.

11.1.4.2 Decreasing Bioavailability of Toxic Contaminants

Reducing the activity of hazardous materials can apparently also be achieved by humic matter by decreasing the bioavailability of contaminants. Possibilities to this effect have been presented recently by Perminova et al. (1997), who show that humic matter restricts the bioavailability of polyaromatic carbon compounds to aquatic organisms. Algae, treated with phenanthrine (PHEN) and fluorenthene (FL), have been tested as a food source for the crustacea *Daphnia magna*. In the determination of the feeding activity of the organism, a substantial decrease in algae cells has been observed with the application of humic matter, interpreted by the authors above as an indication of increased consumption of the algae by *Daphnia*. The authors have associated this with the formation of humo-PHEN and humo-FL algal complexes, which apparently has decreased in this way the bioavailability of the toxic organics, or, in other words, the alga-humo-Phen complexes and alga-humo-FL complexes were not consumed by the crustacean. A decrease in phytotoxicity due to humic treatments of the pesticides atrazine and glean to plants has also been obtained and ascribed similarly by the authors above as a decrease in pesticide bioavailability to plant attack.

As discussed earlier, a pesticide residue may also be detoxified by incorporation as an integral part of the humic molecule. However, it should be emphasized here that the use of organic waste in the synthesis of new humic-like substances is not limited to pesticides only, but also includes a variety of other industrial organic wastes. For example, aromatic amines from the production of dyes and pharmaceuticals may be released in the environment because of incomplete treatments of industrial wastewater. Detoxification of these harmful organics has also been assumed to occur by their incorporation in the humic molecule (Thorn et al., 1996).

11.1.4.3 Effect of Different Origin of Humic Acids

New information from more recent studies on biodetoxification of xenobiotics seems to suggest that humic substances of different origin may have different impacts. The effect of humic substances isolated from different soils is noted by Perminova et al. (2001) to vary greatly on detoxification of atrazine and trifluralin. Humic acid from chernozems (mollisols) is reported to be the best at decreasing the toxicity of the two herbicides, whereas the least detoxifying effect has been observed for humic acids from podzols (spodosols). As indicated earlier in this book, a number of scientists have suspected the presence of such differences in humic compounds. Kononova (1966) is, for example, one of the scientists who has hinted at possible differences between humic acids from peat (anaerobic environment) and normal (aerobic) soils. No specific data, other than conjecture, can be presented up until now from research or the literature to support this contention. However, the data from Perminova and coworkers above provide few but concrete leads for confirming the presence of differences in humic acids from different origin. Nevertheless, more information is required to firmly establish that different soils may contain humic acids different in chemistry and behavior.

11.1.5 Degradation of the Soil Ecosystem

Soil degradation is not just an agricultural issue but also an environmental issue. It can be distinguished into (1) physical soil degradation, (2) chemical soil degradation, and (3) biological soil degradation. Physical degradation is exemplified by deterioration of the physical properties of soils, which mostly is a result of destruction of soil structure because of severe losses of soil organic matter and in particular of humic acids (Bongiovanni and Lobartini, 2006). Chemical degradation, for example, due to loss of nutrients by

leaching and percolation and due to deterioration of soil hygiene by chemical contaminants, can also be prevented by proper maintenance of soils with respect of their humic reserve and supply. The huge adsorption, ion exchange, and buffer capacity of humic matter should not be underestimated as prime factors in combating chemical degradation. Biologically, humic acids commonly foster the development of an array of microorganisms beneficial for a healthy soil ecosystem. For more details on each of these processes, reference is made to Tan (2009).

The processes of soil degradation are in fact natural processes, and all soils will be affected by them. In nature, they are very slow processes, and can perhaps be considered a process of aging or growing old in terms of animal, plant, and human life. However, because of human interference, the degradation process in soils is suddenly accelerated, which is often reflected by a process that we call *accelerated erosion*. Accelerated and natural erosion can take different forms, but they have generally the same results; that is, leaching and carving the landscape of the earth. The only difference is that it will take natural erosion millions of years to carve the landscape of the earth, whereas accelerated erosion is able to do it, if not within several years, within a person's life span.

The accelerated erosion process starts perhaps as soon as the soil is exposed, because of the rapid destruction of soil organic matter. The exposed soil is subject to attack by rain and wind. In the absence of soil organic matter, the impact of raindrops destroys soil aggregates and disperses clay particles, which clog the soil pore spaces. Runoff, created during heavy and light rains, may carry the individual loose sand, silt, and clay particles, and the latter will choke rivers and lakes. In turn, this process reduces the storage capacity of those lakes and rivers. Under dry conditions, the wind will blow dust particles in the air, also reducing air quality. When the surface soil has been stripped off by accelerated erosion, the exposed subsoil, which is often rich in iron, especially in the tropics, may harden and form an iron pan. This pan formation inhibits further vegetative growth.

11.1.5.1 Controversy of Shifting Cultivation

The method of *shifting cultivation* is often claimed to result in immense degradation of the soil's ecosystem and the environment. It is practiced usually in humid tropical regions where extensive areas of lands are available for cultivation. Considered by many scientists as a primitive method and erroneously identified as synonymous with the *slash-and-burn technique* in

crop production (Glaser et al., 2002), the practice of shifting cultivation has recently attracted worldwide attention due to an allegedly massive deforestation and ecological damage. By definition, shifting cultivation includes complex cycles of processes that allow deforested lands to remain fallow for some time after cultivation to recover adequately before being slashed and cultivated again. The system encourages at the end of the cropping cycle the regrowth of a secondary forest, which hence will, on the contrary to what many believe as an environmental disaster, result in minimal ecological damage. On the other hand, slash and burn, a method by itself, is only part of it. The slash-and-burn technique is a system also practiced in humid temperate forested regions without the necessary cycles of fallow and reforestation common in shifting cultivation (Tan, 2008, 2009). Slash and burn is applied in shifting cultivation out of necessity for selective burning of trash, leaves, and twigs to clean the area and to quickly start growing the food crops necessary for living, but normally by salvaging the bulk and larger tree trunks for timber and firewood. The CO₂ and other carbonaceous gases produced are always blamed for causing global warming and harmful climatic changes. It is instead the massive deforestation and burning for clearing lands for establishing of, for example, huge oil palm plantations in Southeast Asia and other purposes in the Amazon forest of Brazil that causes the issues in CO₂ emissions. In shifting cultivation, the ash produced from burning containing all the essential nutrients functions similar to a one-time application of fertilizers. The charcoal, when present as "activated" charcoal, may possess a high adsorption or ion exchange capacity, which at the start may be loaded with plant nutrients. Charcoal by itself is essentially composed of carbon elements only and is not capable of enhancing soil fertility as perceived by some scientists. Charcoal, soot, and other types of carbonized materials have been reported for maintenance of high soil organic matter content and high soil fertility of *anthropogenic soils* of the Brazilian Amazon basin, called the *Terra Preta soils* of the pre-Columbian settlements (Glaser et al., 2002). Black carbon, or carbon black, the more common name, is cited as a possible source for formation of humic acids (Haumaier and Zech, 1995). Another reason, which is backed by scientific research, is that the burning process may have created intense heat capable of producing *carbon nanotubes*, which may explain the beneficial effect of carbon black, charcoal, soot, and other burned carbon residues of this kind in making the soil fertile, an issue examined in detail in Chapter 3. In 2009, scientists from the University of Arkansas have treated tomato seeds with carbon nanotubes, resulting in enhanced germination and substantial increased growth

of tomato seedlings (Khodakovskaya et al., 2009). This has created a lot of excitement among several U.S. humate-producing companies trying to promote carbon nanotubes as some form of *superfertilizers*. As discussed in Chapter 3, the effects of carbon nanotubes on tomatoes are conspicuously similar to the effects observed earlier in 1979 by Tan and Nopamornbodi (1979a) of their humic acid treatments on germination of seeds and growth of corn (maize) seedlings.

11.1.5.2 Issue of Soil Aggregation

The notion exists that the degradation processes discussed above can be prevented by encouraging interaction to occur between soil inorganic and organic particles, thereby forming stable soil aggregates that are less susceptible to physical, chemical, biological, and weather attack. The interaction process, known as *soil aggregation*, sometimes called *granulation*, is often associated with the formation of soil structures. The effect of humic substances increasing soil aggregate stability has been an issue for scientific research by Piccolo et al. (1997), who concluded humic acids to be capable of increasing the structural stability of soils. A recent review by Bronick and Lal (2005) supports the present author's idea above that the physical health of the soil's ecosystem and the environment is affected by the formation of stable soil structures. The degree of soil stability depends to some extent on aggregation of soil particles from rearrangement, flocculation, and cementation by soil organic substances and, in the author's opinion, in particular by humic acids. Although the aggregation process of soil particles is strongly influenced by environmental and especially biological factors, good stable soil structures are noticed only to be formed in the presence of large amounts of soil organic matter. For instance, soil aggregation resulting in stable crumb and granular structures in mollisols occurs usually in the presence of abundant amounts of humic substances, creating the clay–humic acid chelates by Ca bridging. The granular structures in andosols are additional examples of stable aggregates formed by Al bridging of clay and humic acid particles. Increased aggregation will, in turn, increase pore spaces and decrease bulk density, factors beneficial for plant growth. Continuous cropping, however, will decrease the amount of organic matter in soils, and lowering the organic matter content is expected to decrease soil aggregation. Especially the macropore spaces will be destroyed by the destruction of soil structure, which will harmfully affect water percolation and soil aeration.

11.2 Importance in Industry

Humic matter can be applied for a variety of purposes in industry, but unfortunately not much has been published. Most of the information in industry is either patented or kept highly classified. This is, of course, understandable, in view of the fierce competition in the industrial marketplace that can adversely affect the livelihood of the company. However, such an attitude is a disadvantage for the advancement of science and technology. Fortunately, the situation is substantially better in the agricultural and pharmaceutical industries, where scientists are more at liberty to publish their discoveries and inventions. Nevertheless, several applications of humic substances in industry are mentioned in the older literature. Because of its brown to black color, humic matter has attracted attention for possible use as a staining agent. Patents have been obtained for the production of wood stains and tanning material of leather from humic substances, apparently in the United States or by Japanese, Russian, and other overseas companies, as quoted from a review by MacCarthy and Rice (1994). The color of humic substances also finds application in the ink industry, and for coloring ceramics, whereas its dispersing property is important for use in drilling fluids and in paints. The usefulness of humic acids in the production of ink and its importance for removing industrial boiler scales have been reported in the older literature (Steelink, 1963, 1967). Humic matter is also believed to be essential in keeping smectite (montmorillonite) slurries in emulsive form. These slurries are used as lubricants in drilling heads; hence, it is necessary to prevent them from becoming aggregated and hard.

11.2.1 Production of Humo-Silicones

Today complex formation between silicic acid and organic compounds finds application in industry in the production of *silicones*. The general formula of silicone is R_2SiO_2 , in which R is an organic radical. Usually the radical is a simple organic compound, a methyl group, CH_3 , or an ethyl group, C_2H_5 . The suggestion is presented here that humic matter can also be used as a radical. These silicones can be linear or cyclic in structure (Figure 11.1), and silicones composed of cross-linked silica polymers are also possible. Depending on the molecular complexity, silicones have the appearance of oily, greasy, or rubber-like substances. Consequently, they are used as lubricants, hydraulic fluids, and electrical insulators. Antifreeze for automobiles

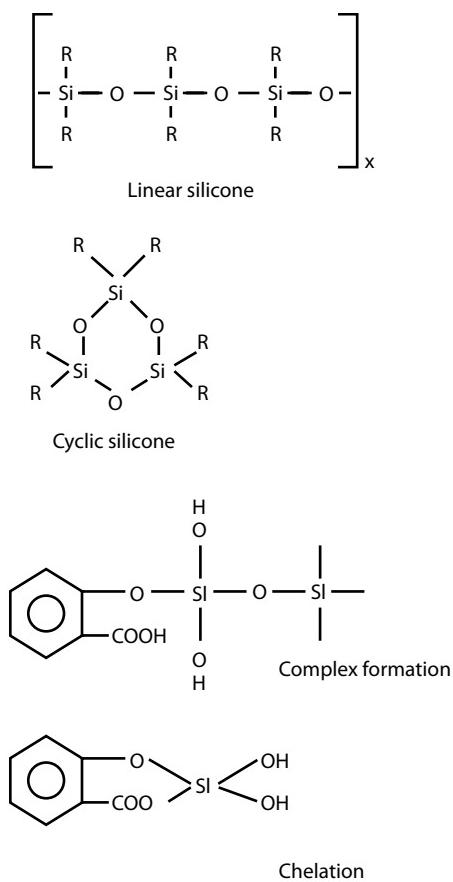


Figure 11.1 Structure of silicone. Top two: Linear and cyclic silicone. Bottom two: Proposed silicone produced from humic acid and silicic acid.

contains silicone for lubricating the water pump. In medical science, silicone finds application as filling material and implants in human bodies. The controversial use of silicone for breast implants has received a lot of attention in the media. Whether the use of humic matter in silicones will affect their behavior is a matter for future investigation, and humic acid is thus far known to be nontoxic.

11.2.2 Production of Bio-Superphosphates and Other Agrochemicals

The metal chelation properties of humic matter also find application today in the fertilizers industry. Considerable efforts have been spent in Europe to

produce and market fertilizers treated with humic acids. It is believed that mineral fertilizer grains coated with humic substances reduce the entropic action of the soil, enhancing in this way the fertilizer efficiency (Savoini, 1986). Humus treated with ammonium hydroxide solution, called nitrogen-amended humus by MacCarthy and Rice (1994), has been used as a fertilizer and was found to have a stimulating effect on plant growth. However, some caution should be exercised because, according to the authors above, large applications of NH_4OH cause the mixture to become harmful. The chemical reaction between ammonium and humus is called NH_3 fixation by Stevenson (1994). It is not related to fixation of ammonium by expanding clays. This capacity to adsorb NH_3 has been known for some time, and is exhibited in fact by an array of organic compounds, such as lignin, peat, sawdust, and other organic residues, e.g., corn cobs. Over the years, a number of patents have been given for the production of biofertilizers from treatment of these residues with NH_3 .

Since humic matter is also a powerful chelator of phosphates, the possibility arises that humic acids can find application in the manufacture of *bio-superphosphates*. In the production of superphosphate fertilizers, the sulfuric acid needed to solubilize P from rock phosphate can perhaps be replaced by humic acids. Although not particularly strong, the acidic properties of humic acids can rival those of sulfuric acid in making part of the rock phosphate soluble. In view of the excellent record of humo-phosphate chelates in stimulating plant growth, the resulting bio-superphosphate or humo-superphosphate fertilizer can prove to be superior to its inorganic counterpart.

In Europe, iron, zinc, manganese, and copper chelates have also been produced in the form of capsules or pellets for use in alleviating micronutrient deficiencies in plants. The humo-metal complexes provide for easy transport and distribution of the micronutrients. Savoini (1986) indicates that these chelates exhibit a slow and gradual solubility in water in addition to a strong resistance to degradation by light and biophysical attack. They can also be mixed with fertilizers since the slow solubility of Fe chelate, for example, makes the Fe humate compatible for mixing with a wide range of fertilizers. Humo-micronutrient chelates in liquid form applied as foliar sprays have proven to be more efficient in controlling micronutrient deficiencies than the plain micronutrient elements alone.

The application of humic matter is not limited to production of fertilizers, but as with the bio-N fertilizers, the manufacture of biopesticides is open for consideration and is already appearing in the near horizon.

Adsorption of pesticide by humic matter is considered by Stevenson (1994) to be a key factor in determining its bioactivity and other behavioral properties, e.g., biodegradability. This is a controversial issue since in one aspect the biopesticide or humo–pesticide complex can become more efficient in attacking its target, but on the other hand, such a complex can stimulate its persistence in soils and increases the danger to nontarget materials.

11.2.3 Production of Commercial Humates

Today humic acids are applied in the production of a variety of compounds for use in soils and agriculture. Some of these applications have been discussed in the previous section. In the United States and several other countries, the kinds of humic products in question are called by many of the producers *commercial humates* or plain *humates*. Canadian and Australian producers call them, on the other hand, *humalites*. Scientifically, “humates” is a proper name since they are, in essence, the salts of humic or fulvic acids. Often the name humate is used by the companies interchangeably with *leonardite*, a *lignite* also called *oxidized lignite*, which are the original materials. This raw material—formed by weathering of organic material for centuries and deposited in the form of soft coal, known usually as lignite or leonardite and mudstone—constitutes extensive commercial humate resources of New Mexico, Utah, North Dakota, Wyoming, Louisiana, Texas, Saskatchewan, and Alberta in North America. Humate-rich sand and sandstone also occur in Florida and along the Gulf Coast (Swanson and Palacas, 1965). The deposits above are usually mined by front-end loaders and stock-piled to dry before crushing and screening. The screened material is bagged and shipped in bulk, or used for custom blending or liquefied. The humate resources in North Dakota are reported to be 7.4 megaton with a production of 28.1 kiloton per year, whereas those in the New Mexico San Juan basin amount to 5 to 7 megaton with a production of 18 kiloton per year (Hoffman et al., 2013). As technical grade chemicals, these humates are seldom in purified forms, as are also the fertilizers. Purification to make them analytical-grade reagents is unnecessary for their use in field conditions, and may even increase the cost considerably, making them unaffordable for farmers and growers alike.

Most scientists in the past considered humates to be “dirt,” hardly worth research attention, and many today still dislike them. However, like it or not, these commercial humates are here to stay, either because of their observable benefits to soil and plants, or to their market persistence. They appear

now to be a multimillion-dollar business venture, and the tendency is that their importance will increase in the near future. Companies producing and marketing humates are noticed to mushroom worldwide. They come and go, because some seem to be out of business after a duration of a few short years due to business competition and/or other reasons.

11.2.3.1 Origin and Types of Commercial Humates

Companies—such as Dinosoil, Alpine, Texas; Earth Greens, Dallas, Texas; GreenSense Humate, Garland, Texas; Midwest BioAg, Blue Mounds, Wisconsin; CropChem, Decatur, Illinois; American Colloid Company, Chicago, Illinois; Humate International, Jacksonville, Florida; Home Hydroponics, Ontario, Canada; Biotechnica, Reading, United Kingdom; Australian Humates, Melbourne, Victoria, Australia; Humin Tech, Dusseldorf, Germany; Pharmawerk, Weinböhla, Germany; TEHUM S.R.O., Czech Republic; Chemapex, Praha, Czech Republic; and AMAGRO, Saint Petersburg, Russia—are now offering a wide range of humates. The listing of companies above is not an endorsement of the particular company or its product, but is only a statement of their current presence in the world market. Many other companies not listed above are also, at present, offering similar humates. Several large producers of humates are also available in China. The humates are generally marketed in solid or liquid form under a variety of generic names, e.g., dinosoil, humate, Menefee humate, enersol, humate Ls/Ag, liquid gold humate, biohumate, organic humate, K humate, humigel, and the like.

Most of the produced humates are from lignite-based humic material. Some are in the form of powdered or granulated lignite only (Lobartini et al., 1992; Burdick, 1965), whereas several are lignite humic acid in liquid form dissolved in NaOH, KOH, or NH₄OH. A few of them constitute mixtures of lignite humic acid and seaweed or other marine life residues, whereas many more may be fortified by the addition of essential macronutrients and micronutrients for plant growth. Less known are the humates produced by inoculation of lignite or lignite–humic acid with microorganisms, or humates mixed with bacteria, fungi, and *Actinomyces* species.

Depending on the extraction reagents used, the humates are either Na, K, or NH₄ humate. Because of the absence of a proper purification process, the commercial humates are high in pH, and pH values of 10–12 are not uncommon. The K and NH₄ humates are expected to be relatively high in K and NH₄, respectively, and, by origin, humic acids already

contain 4% to 5% N, small amounts of S, and perhaps traces of P. The extra amount of macronutrients and micronutrients added to fortify the humates are sometimes quite large. For instance, Humate Fertilizer PK and Humate Fertilizer N, offered by Chemapex, Praha, listed contents of 14% P_2O_5 , 12% K_2O , and 12% N, whereas the organic humate from Australian Humates listed 24 mg/L B, 200 mg/L Fe, and 40 mg/L Mn. The very small amounts of micronutrients required by plants is perhaps the reason why the fortified humates may in some instances yield beneficial results, and in other instances no results. To erase the issue of mixed results, a thorough research investigation is perhaps required for testing and determination of the proper balanced content of micronutrients in the humates.

11.2.3.2 Issue of Mixing with Microorganisms

Of interest to note are also the humates produced by mixing or inoculating with a variety of microorganisms and organic compounds, referred to as *biosystems*, *bactolife*, or *biostimulants*. Several of the “biosystem” microbial products specify the inclusion of a broad spectrum of bacteria, fungi, and actinomycetes with the humate. It may perhaps work in sterile environments; however, pending more information from scientific research, some caution should be exercised in introducing an assortment of foreign organisms into the soil. In agricultural operations, it is common to add an organism for a known purpose, such as adding *Rhizobium* strains for inoculation of legume plants to enhance biological N₂ fixation, or by applying mycorrhizal fungus, e.g., *Pisolithus tinctorius*, to pine seedlings to improve their growth (Tan, 2009). Seldom is an indiscriminate application of a large variety of microorganisms warranted for a general purpose since the soil may already contain most of the organisms. The organisms foreign to the soil may either be killed by the indigenous microbial population or they may cause damage to the latter.

11.2.3.3 Issue of Mixing with Saponin

Mixing humate with *saponin* as in the case of Biohumate of Australian Humates is perhaps more warranted than mixing with an assortment of live microorganisms for undetermined purposes. Saponin is believed to function as a biostimulant, but this reagent is in general known to reduce surface tension, like soap, hence will add the surfactant property of soap to the humate, which is of utmost importance for humate applications as foliar sprays. In biochemistry, saponins are grouped with the *glycosides*, which

play a role in blocking the activity of toxic substances in the plant tissue so that plant cells are not injured. However, some of the saponins are toxic to some animals and have been used in Africa, Asia, and South America to catch fish in streams and lakes. The plants containing saponins are beaten to pulp upstream in the river, and the poisoned fish, edible for humans, rise downstream to the surface.

11.2.3.4 Issue of Humates as Fertilizers

The opinion of many companies that humates can be considered as fertilizers raises a lot of questions since most soil scientists prefer to classify them as soil amendments. By origin, humic acids do not carry nutrient elements in the same amounts as fertilizers do. Their N content, as stated above, is also too small to qualify them as fertilizers. The use of KOH and NH₄OH in the production of humates will indeed increase their K and N contents, respectively, but not to the extent that they can easily be called fertilizers. Nevertheless, plants have been noticed to respond quickly to the application of these K or NH₄ humates. The NH₄ humates may perhaps be of more advantage to plant growth than the K humates, due to the more visible effect of N in causing a healthy appearance in plants by producing greener leaves. The effect of K humates is expected to be less conspicuous since K affects carbohydrate production, leaf-tip growth, and some root formation. Not much research has been conducted in this respect.

Despite all the above, the definition of a fertilizer states that a *fertilizer* is any material applied to soils to improve the growth, yield, quality, and nutritive value of crops (Tisdale et al., 1993; Jones, 1982; Tan, 1981). According to such a concept, commercial humates should fall in the category of fertilizers owing to the many claims that they enhance germination, root development, nutrient uptake, growth, and yield of plants. Cow manure, stable manure, compost, and the like are called organic fertilizers; hence, why should the humates not be considered as such? The only difference is that the commercial humates do not contain N, P, K, Ca, Mg, and other plant nutrients in the same amounts as do cow and stable manure, unless they are fortified with these nutrients during the manufacturing process.

Other claims that humates improve microbial activity, soil structure development, and water-holding capacity are also scientifically well founded, as is the action of humates as chelators. Some humates are shown to contain "appreciable" amounts of cytokinins, gibberelins, and auxins, empowering them consequently with the capabilities of growth hormones. This is

especially of importance with humates produced from or mixed with seaweeds. Whether the latter will be found to be true or not is a matter for investigation, since, as discussed in Chapter 5, some truth may be present in all these assertions. Hormones can be released by decomposition from plant materials and incorporated in the humic molecular structure during the humification process. Other growth-promoting substances, such as vitamins, are believed to also be included in the synthesis of humic matter. These then are the reasons for the growth-stimulating effects often reported for humic substances.

11.2.3.5 Rate of Application

A lot of research is apparently still needed to establish the rate of humate application since the latter shows considerable variations from one to another brand of humate. Amounts of 100 to 300 kg/ha of the solid form of humates have been suggested for application in field conditions by U.S. companies, but 600 kg and 5 tons/ha have been mentioned by Australian Humates.

The liquid form of humates provides fewer problems in application, and according to several scientific studies a rate of 400 to 600 mg/L humic acid is optimum. Higher rates of ≥ 1000 mg/L can be beneficial for one, but harmful, although not toxic, for other crops (see Chapter 9). This liquid humate refers, in fact, to a humate product produced by mixing lignite (or leonardite) with a determined amount of strong base—an aqueous solution of NaOH, KOH, or NH₄OH—until a 6% to 12% solution is produced with a pH of > 9.0 (Zimmer, 2004). The black aqueous alkaline solution, marketed in the United States in gallon jugs, is commonly mixed with liquid nitrogen fertilizers or with herbicides before field applications. In crop production, generally 1 to 3 gallons/acre (1 gallon/acre = 9.5 L/ha) is allegedly required depending on the amount of N fertilizers needed by the crops. Many farmers use this liquid humate also in fertigation.

11.3 Importance as Pharmaceuticals

11.3.1 Balneotherapeutic Use of Peat

The idea of using humic matter in the pharmaceutical industry is derived from a number of reasons. The oldest reason comes perhaps from peat,

which has been applied for a long time in Europe for its healing properties. The therapeutic use of peat baths, called scientifically *balneotherapy* or the balneotherapeutic use of peat, is according to Klöcking and Helbig (2005) the most significant application of humic substances in medical sciences. The healing effect of mud or peat was already recognized in the old days in Babylonia and in the Roman Empire. As health clinics were developed over time, peat or mud baths have been offered since the early nineteenth century for the therapy of *gynecological* and *rheumatic* diseases. Even today mud baths are still popular in many European health spas and clinics. In our modern cosmetic industry, face muds are offered at expensive prices for curing or removing old skin. The healing properties have been currently traced to humic acid, the dominant component of peat. Besides the popular mud or peat baths, drinking cures were also developed in the 1960s for gastric, intestinal, or hepatic ailments. A summary of today's applications of peat therapy at various modern health clinics in Germany, as reported by Klöcking and Helbig (2005), includes several types of rheumatism, osteoporosis, osteoarthritis, gout, eczema, psoriasis, and more.

11.3.2 Humic Substances in Pharmacology and Medicines

Today, it is the adsorption, chelation reactions, dispersion or emulsification properties, and other attributes of humic matter that attract the interest of medical science and the pharmaceutical industry. The potential for use of humic acids as antiviral, anti-inflammatory, estrogenic, profibrinolytic, and anti-coagulatory agents has been under serious investigations for some time at the Medical School in Erfurt, Germany. With sodium humate, instead of peat, the analgesic, anti-inflammatory and lipid modulatory effects of humic acids are scientifically confirmed in various clinical tests (Klöcking and Helbig, 2005). Studies on the treatment of viruses using humic substances started in the early 1960s, after the successful fight with foot-and-mouth disease by peat. Clinical research with the influenza-A virus and herpes simplex virus type 1 showed humic acid to be effective against the two viruses. This has apparently also led to the development of a lipstick containing humic acids to prevent recurrences of UV radiation-induced herpes (Klöcking et al., 2013).

A successful treatment of thrombophlebitis is credited earlier by Klöcking (1994a, b) to the anticoagulatory property of humic acid. In a more recent investigation, the author above reinforces his contention by demonstrating humic acid at dilute concentrations (0.5–6.2 µmol/L) to function as a potent

inhibitor of *thrombin* (Klöcking, 1997). Its antiviral potency is revealed by Thiel et al. (1977), who claim to have successfully treated herpes simplex virus infection with dilute concentrations (0.5 mg/L) of ammonium humate. Clinical tests in Hungary show, in addition, possibilities for humic acids to be used in cancer therapy and in healing crush, cut, and especially burn wounds (Jurcsik, 1994).

In Russia, a medicine prepared from peat extracts, called *torfot*, is used at the Ukrainian Institute of Eye Diseases at Odessa as a topical treatment for myopia, opacification of the cornea, and early retinal degeneration (Fuchsman, 1980). Perhaps it can also prove to be useful one day for retarding or slowing the growth of cataracts.

11.3.3 Humic Acids in Veterinary Medicine

In veterinary medicine, humic acids have been used because of their huge cation exchange and chelation capacities. The organic compounds are usually applied as prophylaxis, including therapy of diarrhea and as reagents for other gastrointestinal ailments or as antidotes against accidental intake of harmful toxic metals. Intoxication of rats due to consumption of the heavy metals, Cd and Pb, is reported to be controlled by the application of low concentrations (0.1%) of humic acids in their food supply (Ridwan, 1977). This opens possibilities for use of humic acids in treatments of other stomach ailments in the line of the over-the-counter medicine called Kaopectate or Pepto-Bismol for humans. The adsorption capacity of humic matter is far greater than that of kaolinite, and thus far humic substances are not known to be toxic to soil organisms. Fish and other aquatic organisms are noticed to thrive prolifically in black water or humic water environments, as indicated earlier. Detailed clinical tests in European hospitals on the safety and toxicology of humic acids have shown the lethal dose to be extremely high, exceeding 10 g/kg body weight of animals under investigation.

11.3.4 Prospects of Humic Acids as Medicine in the United States

The application of humic acids in medicine and in the pharmaceutical industry in the United States is currently lagging far behind that in European countries. Mention should perhaps be made of the presence of Morganics Inc., Scottsdale, Arizona, a company that has just started to recognize fulvic acid as a powerful antioxidant (personal communications from Craig Ricketts, CEO). However, its product with an effect closest to

humate is perhaps Earth Force, which Morganics claims to act as a biological activator for flowering and potted plants and also enhance seed germination. Once again it should be emphasized that no endorsement whatsoever is presented here.

11.3.5 Issue of Mutagenesis and of Blackfoot Disease

Most of the reports show beneficial effects of humic acid on human and animal health, and only on a few occasions are they allegedly harmful. Mixed reports are presented for the potential of humic acid in causing mutagenesis, or in plain language, "humic acid is suspected to induce mutation." This opinion surfaces from the chlorination of drinking water containing minute amounts of humic substances. One of the resulting chlorinated intermediate products—3-chloro-4-(dichloromethyl)-5-hydroxy-(5H)-furanone—is known to cause mutagenicity (Klöckling and Helbig, 2005). However, thus far, no mutagenesis has been observed due to the great instability of furanones.

Another possible harmful effect implicating humic acids is the endemic blackfoot disease (BFD) in Taiwan, a peripheral vesicular disorder allegedly due to drinking water containing humic substances (Jean, 2006; Cheng et al., 2003). Artesian or well water used as drinking water in Taiwan contains, in some cases, humic substances and/or arsenic. Preliminary studies indicate that it is perhaps the arsenic content that is the prime reason for the BFD rather than the humic acid concentration (Klöckling and Helbig, 2005). Moreover, clinical toxicological tests in European hospitals indicate that the harmful dose of humic acids is extremely high, with a lethal effect exceeding 10 g/kg bodyweight, as stated above.

Nevertheless many believe that humic acids occurring naturally or produced artificially have the potential to become effective drugs for healing a variety of ailments (Klöckling and Helbig, 2005).

11.3.6 Controversy Surrounding the Name Humate-P

A final note in the medicinal application of humic acids is the product Humate-P, a biological compound approved by the U.S. Food and Drug Administration in 1999 (FDA Talk Paper, T99-15, April 1, 1999) for therapy of a clotting disorder. Humate-P has been cleared for treatment of patients with hemophilia A and/or von Willebrand disease, a bleeding disorder. The reasoning for choosing such a name is everybody's guess in soil science since

humate-P is neither a humic acid derivative nor has it any relation to soil humic matter. The P does not stand for phosphorus but for *pasteurization* needed for sterilization of the product. It is a purified product derived from pooled human blood plasma, hence should better carry the name Plasma-P or Plasmate-P. Using the name Humate-P only brings confusion to humic acid chemistry and science, unless the plasma can be proven to contain humic acids.

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Appendix A: Greek Alphabet

| <i>Greek Letter</i> | <i>Greek Name</i> | <i>Greek Letter</i> | <i>Greek Name</i> |
|---------------------|-------------------|---------------------|-------------------|
| A α | Alpha | N ν | Nu |
| B β | Beta | $\Xi \xi$ | Xi |
| $\Gamma \gamma$ | Gamma | O \circ | Omicron |
| $\Delta \delta$ | Delta | $\Pi \pi$ | Pi |
| E ε | Epsilon | Rho | Rho |
| Z ξ | Zeta | $\Sigma \sigma$ | Sigma |
| H η | Eta | Tau | Tau |
| $\Theta \theta$ | Theta | $\Upsilon \upsilon$ | Upsilon |
| I ι | Iota | Phi | Phi |
| K κ | Kappa | Chi | Chi |
| L λ | Lambda | $\Psi \psi$ | Psi |
| M μ | Mu | $\Omega \omega$ | Omega |

Appendix B: Atomic Weights of Major Elements in Soils

| <i>Element</i> | <i>Symbol</i> | <i>Atomic Number</i> | <i>Atomic Weight</i> |
|----------------|---------------|----------------------|----------------------|
| Aluminum | Al | 13 | 027.0 |
| Antimony | Sb | 51 | 121.8 |
| Argon | Ar | 18 | 039.9 |
| Arsenic | As | 33 | 074.9 |
| Barium | Ba | 56 | 137.3 |
| Beryllium | Be | 04 | 009.0 |
| Bismuth | Bi | 83 | 209.0 |
| Boron | B | 05 | 010.8 |
| Bromine | Br | 35 | 079.9 |
| Calcium | Ca | 20 | 040.1 |
| Carbon | C | 06 | 012.0 |
| Cesium | Cs | 55 | 132.9 |
| Chlorine | Cl | 17 | 035.5 |
| Chromium | Cr | 24 | 052.0 |
| Cobalt | Co | 27 | 058.9 |
| Copper | Cu | 29 | 063.5 |
| Fluorine | F | 09 | 019.0 |
| Gallium | Ga | 31 | 069.7 |
| Germanium | Ge | 32 | 072.6 |

| <i>Element</i> | <i>Symbol</i> | <i>Atomic Number</i> | <i>Atomic Weight</i> |
|----------------|---------------|----------------------|----------------------|
| Gold | Au | 79 | 197.0 |
| Helium | He | 02 | 004.0 |
| Hydrogen | H | 01 | 001.0 |
| Iodine | I | 53 | 126.9 |
| Iridium | Ir | 77 | 192.2 |
| Iron | Fe | 26 | 055.9 |
| Krypton | Kr | 36 | 083.8 |
| Lanthanum | La | 57 | 138.9 |
| Lead | Pb | 82 | 207.2 |
| Lithium | Li | 03 | 006.9 |
| Magnesium | Mg | 12 | 024.2 |
| Manganese | Mn | 25 | 054.9 |
| Mercury | Hg | 80 | 200.6 |
| Molybdenum | Mo | 02 | 095.9 |
| Nickel | Ni | 28 | 058.7 |
| Nitrogen | N | 07 | 014.0 |
| Oxygen | O | 08 | 016.0 |
| Phosphorus | P | 15 | 031.0 |
| Platinum | Pt | 78 | 195.1 |
| Potassium | K | 19 | 039.1 |
| Radium | Ra | 88 | 226.1 |
| Radon | Rn | 86 | 222.0 |
| Rhodium | Rh | 45 | 102.9 |
| Rubidium | Rb | 37 | 085.5 |
| Selenium | Se | 34 | 079.0 |
| Silicon | Si | 14 | 028.1 |
| Silver | Ag | 47 | 107.9 |
| Sodium | Na | 11 | 023.0 |

| <i>Element</i> | <i>Symbol</i> | <i>Atomic Number</i> | <i>Atomic Weight</i> |
|----------------|---------------|----------------------|----------------------|
| Strontium | Sr | 38 | 087.6 |
| Sulfur | S | 16 | 032.1 |
| Tantalum | Ta | 03 | 180.9 |
| Tellurium | Te | 52 | 127.6 |
| Thallium | Tl | 81 | 204.4 |
| Thorium | Th | 90 | 232.1 |
| Tin | Sn | 50 | 118.7 |
| Titanium | Ti | 22 | 047.9 |
| Tungsten | W | 74 | 183.9 |
| Uranium | U | 92 | 238.0 |
| Vanadium | V | 23 | 050.9 |
| Xenon | Xe | 54 | 131.3 |
| Yttrium | Y | 39 | 088.9 |
| Zinc | Zn | 30 | 065.4 |
| Zirconium | Zr | 40 | 091.2 |

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